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FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER
899-59399

U S APPLICATION NO (If known, see 37 C F R § 1 5)
09/890806

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/02740	2 February 2000	2 February 1999

TITLE OF INVENTION
INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS

APPLICANT(S) FOR DO/EO/US
David C. Johnson, Roman Tomazin, Jessica Boname, Nagendra R. Hegde

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U S C § 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U S C. § 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U S C § 371(c)(2))
   a ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S C § 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U S C § 371(c)(3))
   a ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
   b ☐ have been transmitted by the International Bureau
   c ☐ have not been made, however, the time limit for making such amendments has NOT expired
   d ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U S.C. § 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S C § 371(c)(4)) (unsigned)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S C § 371(c)(5))

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C F R §§ 1 97 and 1 98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R §§ 3 28 and 3 31 and the Recordal fee of $40 00 is included
13. ☒ A **FIRST** preliminary amendment
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification
15. ☐ A change of power of attorney and/or address letter
16. ☒ Other items or information
    ☒ Sequence Listing (Paper Copy and Diskette)
    ☒ Statement in Compliance

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U.S. APPLICATION NO (If known, see 37 C F R. § 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/890806	PCT/US00/02740	899-59399

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):

Neither International Preliminary Examination fee (37 C.F.R. § 1.482) nor International Search fee (37 C.F.R. § 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... **$1,000.00**

International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... **$860.00**

International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C F R. § 1.445(a)(2)) paid to USPTO..... **$710.00**

International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1 482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... **$690.00**

International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1 482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**

CALCULATIONS (PTO USE ONLY)

ENTER APPROPRIATE BASIC FEE AMOUNT =				**$ 690.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. § 1.492(e)).				**$**
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	38 - 20 =	18	x **$18.00**	**$ 324.00**
Independent Claims	9 - 3 =	6	x **$80.00**	**$ 480.00**
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	**$**
TOTAL OF ABOVE CALCULATIONS =				**$ 1494.00**
☒ Reduction of 1/2 for filing by small entity. Small entity status is claimed for this application.				**$ 747.00**
SUBTOTAL =				**$ 747.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C.F R. §§ 1.492(f)). +				**$**
TOTAL NATIONAL FEE =				**$ 747.00**
Fee for recording the enclosed assignment (37 C.F.R. § 1 21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C F R. §§ 3 28, 3.31). **$40.00** per property. +				**$**
TOTAL FEES ENCLOSED =				**$ 747.00**
			REFUND →	**$**
			CHARGE →	**$**

a. ☒ A check in the amount of $ 747 00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No 02-4550 A duplicate copy of this sheet is enclosed.

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received.

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KLARQUIST SPARKMAN, LLP
One World Trade Center, Suite 1600
121 S.W Salmon Street
Portland, OR 97204-2988

SIGNATURE

Tanya M. Harding, Ph.D.
NAME
42,630
REGISTRATION NUMBER

cc: Docketing

TMH:jlb 08/01/01 62867
Attorney Reference Number 899-59399
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Johnson *et al.*

Art Unit: Not yet assigned

Application No. Not yet assigned

Filed: Herewith

For: INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS

Examiner: Not yet assigned

Date: August 1, 2001

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on August 1, 2001 as First Class Mail in an envelope addressed to: BOX PCT, COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

Tanya M. Harding, Ph.D.
Attorney for Applicant

BOX PCT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

In the Specification, please insert the following header and paragraph on page 1, immediately following the title:

--CROSS REFERENCE TO RELATED APPLICATIONS

This is the National Stage of International Application No. PCT/US00/02740, filed February 2, 2000, and claims the benefit of U.S. Provisional Application No. 60/118,287, filed February 2, 1999. The provisional application is incorporated herein in its entirety.--

By this amendment the specification has been changed to reflect prior related applications. No new matter is added by this amendment.

Respectfully submitted,

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Johnson *et al.*

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CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on August 1, 2001 as First Class Mail in an envelope addressed to: BOX PCT, COMMISSIONER FOR PATENTS, WASHINGTON D.C. 20231.

Tanya M. Harding, Ph.D.
Attorney for Applicant

STATEMENT IN COMPLIANCE WITH 37 C.F.R. § 1.821(f)

BOX PCT
COMMISSIONER FOR PATENTS
Washington, DC 20231

Sir:

In compliance with 37 C.F.R. § 1.821(f), the undersigned declares that the nucleotide and/or amino acid sequences presented in the paper copy of the "Sequence Listing" submitted herewith are the same as the sequences contained in the computer-readable form of said "Sequence Listing."

Respectfully submitted,

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INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS

FIELD

This invention relates to an immunosuppressive agent that can be used to improve persistence of viral vectors and the transgene used in gene therapy, sustain transplanted cells and organs, or to treat autoimmune disorders.

BACKGROUND

The normal mammalian immune system responds to viral infection, or the introduction of "non-self" antigens, *e.g.*, such as antigens present on transplanted tissues, through a variety of complex mechanisms. One such mechanism is the recognition and destruction of such antigens by T lymphocytes (T cells), which selectively kill cells expressing "non-self" antigens, while leaving cells expressing only self antigens unharmed. The ability of T cells to selectively kill non-self cells is related to recognition of major histocompatibility (MHC) complexes that present peptides derived from viral and cellular proteins on the surface of cells. T cells have antigen-binding molecules on their cell surfaces, called T-cell receptors, that react with MHC proteins bearing peptide antigens on the APCs surface.

There are two classes of MHC complexes that mediate different molecular immune responses. MHC class I molecules collect peptides derived primarily from proteins synthesized in the cytosol, and are thus able to display fragments of viral and cellular proteins, normally found inside cells, on the cell surface. MHC class II molecules bind peptides derived from proteins that are often taken up by cells into phagocytic or endocytic vesicles by macrophages and B cells. Internal antigens, produced inside MHC class II-expressing cells, can also be presented by MHC class II proteins. Peptides bound to MHC class I molecules are recognized by CD8+ T cells, while those bound to MHC class II molecules are recognized by CD4+ cells. CD8+ T cells are often cytotoxic T lymphocytes that recognize and remove virus-infected, tumor, or transplanted cells. $CD4^+$ T cells often provide "help" in the form of cytokines and costimulation that is necessary for expansion and activation of CD8+ T cells, and also B cells that produce antibodies. Thus, in the absence of CD4+ T cells, as occurs during human immunodeficiency virus infection of CD4+ T cells, the entire immune system is inhibited.

Although immune recognition of "non-self" proteins is essential to avoid and eliminate infection, the immune response can sometimes be unwanted. Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis or insulin dependent diabetes mellitus, are the result of a pathological immune response against self antigens and T cells are the primary mediators of autoimmunity. Rejection of transplanted organs and tissues are an example of an undesired consequence of normal immunity, which can often result in damage to the transplant. Again, CD8+ and CD4+ T cell responses are important in graft rejection. A related problem is encountered in gene

therapy, where genes are often introduced in vectors (such as viruses) which produce proteins. Both the vector and the protein or transgene can be recognized by the immune system, and cause an immune response to be mounted. The immune response often involves CD8+ and CD4+ T cells and these cells interfere with the function of the vector, and reduce both the effectiveness and duration of the therapy.

Efforts have been made in the past to disarm the immune system using corticosteroids (such as methylprednisilone) or cytotoxic drugs (such as cyclophosphamide), but the widespread and non-specific effects of these treatments often limit their utility or effectiveness. More specific immune modulators, such as FK506, have fewer undesired side effects, but still inhibit the entire immune response, rather than specific immune responses or responses directed to specific tissues. However, more recent efforts have been made to target particular molecular mechanisms of immunity to further refine the ability to modulate specific immune responses.

An example of specific modulation of immunity is shown in U.S. Patent No. 5,750,398, which discloses a method of the treatment and prevention of autoimmune disease by administering or expressing the Herpes Simplex Virus (HSV) immediate early protein ICP47. This protein inhibits the presentation of viral and cellular antigens associated with MHC I proteins to CD8+ T cells. This inhibition was said to be useful to improve the effectiveness of viral gene therapy vectors, by increasing infective persistence.

PCT publication WO 97/32605 disclosed a recombinant human cytomegalovirus nucleic acid encoding a US11 or US2 protein. These proteins, which were said to have specific binding domains for MHC I molecules, downregulated expression of class I MHC genes. The invention included mutating the binding domain of US2 to recognize MHC II but not MHC I molecules, to change the binding specificity and immune function of the protein. However, no evidence was provided that a mutated form of HCMV US2 could bind to or downregulate MHC II.

Although specific downregulation of MHC I or II mediated immunity would be helpful in some situations, it would also be useful to have immune modulators that would downregulate both arms of the cellular immune reaction.

SUMMARY

The present invention concerns use of the human cytomegalovirus (HCMV) protein, US2, or soluble fragments or variants thereof, to inhibit inappropriate immune responses, for example immunity directed at vectors or the transgenes expressed by such vectors, immunity to transplanted cells or tissues, or in autoimmune disorders. The present invention is useful in studying the immune response to vectors, and facilitating delivery of vectors to cells for a variety of purposes. US2 effectively inhibits the MHC class II antigen presentation pathway by causing degradation of two components of this pathway, namely MHC-II-α and DM-α. When these pathway components are destroyed or disabled, cells that normally present antigens via the MHC II pathway can no longer present these antigens to CD4+ T cells. Therefore, cells expressing US2 remain invisible to CD4+ T

cells. In cases of inappropriate immune responses involving CD4+ T cells, it would be very useful to express US2, or a biologically active variant thereof (*e.g.*, a soluble fragment of US2 that retains US2 biological activity), and block the immune responses in specific tissues, without the need to globally inhibit immunity in the entire body.

Although it has previously been found that US2 can inhibit the MHC class I antigen presentation pathway that presents antigens to CD8+ T cells (PCT WO 97/32605), the present invention takes advantage of the unexpected finding that US2 also blocks the MHC II pathway without the necessity of mutating the binding domain. Hence when US2, or a biologically active variant thereof (*e.g.*, a soluble active fragment of US2), is expressed in cells or in tissues, it will have a dual effect of blocking recognition by both CD4+ and CD8+ T cells. In a number of instances of inappropriate immunity (gene therapy, transplantation, autoimmunity) the CD4+ T cell response is dominant, or is required in order for CD8+ T cells to become activated and expand. Therefore, the effects of US2 on the MHC II pathway are important and essential for the protein to function in an effective manner.

PCT WO 97/32605 described mechanistic details of how US2 inhibits the MHC-I antigen presentation pathway. Although that patent suggested that it might be possible to *mutate* or express an altered form of US2 in order to inhibit that MHC-II pathway (and CD4+ T cells), no evidence was presented that this could indeed be done. The present inventors have found that the normal, unmutated form of US2 (or certain soluble truncations thereof) is very active in inhibiting the MHC-II pathway. That PCT publication therefore teaches away from the present invention, because it teaches that US2 must first be mutated or altered in order to inhibit MHC class II.

This finding has allowed the invention to include a method for inhibiting cell recognition by both CD8+ and CD4+ cells, by introducing into a mammalian cell an isolated nucleotide sequence encoding US2, or a variant thereof which maintains the native activity of US2 (*e.g.*, a soluble variant), and wherein the binding domain recognizes both MHC I and MHC II molecules. The binding domain of US2 may be a native binding domain, and not a binding domain that has been mutated to recognize MHC II molecules. In particular embodiments, the nucleotide sequence may be introduced through the use of a viral vector, such as an adenovirus or a retrovirus.

In other embodiments, the invention includes a recombinant expression vector that includes a promoter operably linked to a nucleotide sequence encoding a US2 protein, or a biologically active variant thereof, wherein a binding domain of the US2 protein or variant has not been mutated to recognize MHC II molecules. When present in a mammalian cell, the expression vector inhibits the ability of the cell to present antigens associated with MHC class II proteins to T cells. Inhibition of this ability is useful in the treatment of autoimmune diseases in which CD4+ T cells recognize self MHC II proteins, or in tissue and organ transplantation where CD4+ T cells recognize foreign MHC II proteins. Inhibition of MHC II antigen presentation would also improve the persistence of a virus vector used in gene therapy, such as an adenoviral or retroviral vector, by inhibiting recognition of viral antigens or transgenes presented with MHC class II proteins.

In another embodiment, the vector includes an isolated nucleotide sequence that encodes a protein having the activity of native US2. The vector can be used in methods of treating autoimmune disease, or improving gene therapy, by administering the vector to a subject. In particular embodiments, the vector also includes a nucleotide sequence that codes for a therapeutic product.

Another embodiment is a purified soluble protein having US2 protein biological activity. Such proteins include the US2 binding site for MHC II, and for instance may include the amino acid sequence as shown in about residues 28 through about 143 of SEQ ID NO: 3. Also encompassed in the invention are isolated nucleic acid molecules that encode these purified soluble US2 proteins, and recombinant nucleic acid molecules and cells that include them.

The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description of several embodiments that proceed with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES, METHODS, AND MATERIALS

MHC class II expression is reduced in HCMV-infected cells

Fig. 1A. U373 cells were treated with IFN-γ for 72 hours then left uninfected or infected with wildtype (Wt) HCMV (strain AD169) or a mutant HCMV lacking the IRS1-US11 genes. After 12 hours of infection the cells were radio-labeled with ^{35}S-methionine/cysteine for 30 minutes and the label chased in medium containing excess methionine for 2 hours. MHC class II complexes were immunoprecipitated from cell extracts using MAb DA6.147, specific for class II-α or HCMV immediate early (IE1) protein 86, precipitated using a rabbit polyclonal. Samples were separated by using 14% polyacrylamide gels.

Fig. 1B U373 cells transfected with the MHCII transactivator gene CIITA (U373-CIITAHis) to induce constitutive expression of MHC class II were left uninfected or infected with wildtype HCMV or HCMV mutants lacking the genes indicated for 36 hours. Cells were radio-labeled and class II or IE immunoprecipitated as described in A.

Fig. 1C. U373CIITAHis cells were left uninfected or infected with wildtype HCMV or the HCMV mutant unable to express IRS1-US11 for 36 hours. Total RNA was harvested and separated using agarose gels containing formaldehyde. Cells were disrupted using the TRIZOL reagent (GIBCO, Gaithersburg, MD), according to the manufacturer's directions. The RNA was separated using electrophoresis with a 1% agarose gel containing 2.2 M formaldehyde, transferred to Hybond-N+ membranes and the membranes probed with linearized, ^{32}P-labeled plasmids containing the HLA-DR-alpha, HLA-DR-beta, invariant chain, CIITA, HCMV glycoprotein B (gB), or beta-actin gene. The RNA was transferred to membranes and probed with ^{32}P-labeled plasmids containing the class II-DR-α, DR-ß, Ii, CIITA, glycoprotein B or ß-actin genes.

Fig. 1D Homology tree showing the amino acid identity (%) between classical MHC class I proteins (HLA-A2, HLA-C), non-classical class I HLA-G and MHC class II proteins DR-α, DR-ß, DM-α, and DM-ß (Needleman and Wunsch, *J. Mol. Biol.* 48:443-453, 1970).

Instability of MHC class II-α, but not ß or Ii, in US2-expressing cells

Fig. 2A U373CIITA-His cells were left uninfected or infected with an AdtetUS2 and Adtet-trans using two quantities of virus: 10 PFU/cell of Adtet-trans and 50 PFU/cell of AdtetUS2, or 20 PFU/cell of Adtet-trans and 100 PFU/cell of AdtetUS2 for 12 hours. The cells were radio-labeled with ^{35}S-methionine/cysteine for 1 minute, then the label chased with excess methionine for 0, 15 or 30 minutes before cell extracts were prepared. Immunoprecipitations were then performed under non-denaturing conditions for antibodies specific for the class II complex (DR-α-specific MAb DA6.147), US2 (rabbit anti-US2N), class I heavy chain (rabbit anti-HC), transferrin receptor (TfR) (MAb B3/25), calnexin (rabbit anti-Cxn/c), or grp-94 (MAb 9G10). Alternatively, samples were denatured then immunoprecipitated with antibodies specific for class II-α (MAb DA6.147), class II-ß (MAb HB10A) or invariant chain (Ii) (MAb PIN.1). Proteins were denatured in sodium dodecyl sulfate (SDS) by suspending radio-labeled cells in 1% SDS, 25 mM Tris, pH 7.5, 150 NaCl, 1 mg/ml BSA, and protease inhibitors (SDS lysis buffer) followed by incubation at 95° C for 10 minutes. Extracts were diluted 10-fold in 25 mM Tris, pH 7.5, 150 mM NaCl and 1% Triton X-100. Immunoprecipitations were then performed as described previously (York *et al.*, *Cell* 77:525-535, 1994).

Fig. 2B. Class II DM-α and DM-ß were denatured and immunoprecipitated using the MAb 5C1 and MaP.DMB-C, respectively.

US2 induces degradation of class II-α chains by proteosomes without a cytosolic intermediate

Fig. 3A. U373-CIITAHis cells were left uninfected or infected with Adtet-trans and AdtetUS2 using 20 and 100 PFU/cell, respectively. After 11 hours of US2 expression, proteasome inhibitors (25 uM lactacystin or 35 uM ZL3VS) were added for 1 hour, then the cells were labeled with ^{35}S-methionine/cysteine for 1 minute and the radio-label chased for 0, 20 or 40 minutes. Class II-alpha and class I HC were denatured and immunoprecipitated as described in Fig. 2.

Fig. 3B. U373CIITA-His cells were infected, treated with 35 μM ZL3VS, and then radio-labeled as described in 3A. The cells were then subjected to subcellular fractionation, so that 1,000 X g, 10,000 X g and 100,000 X g pellets and 100,000 X g supernatants were prepared. Radio-labeled cells were homogenized and subjected to subcellular fractionation, as previously described (Wiertz *et al.*, *Nature* 384:432-438, 1996) with the following exceptions. Subcellular pellets were resuspended and denatured in SDS lysis buffer. SDS to a concentration of 1% was added to the 100,000 x g supernatant before heating, then 1% Triton X-100 was added, and the SDS removed by using SDS-

Out (Pierce Chemical Company, Rockford. IL). Membrane pellets or supernatant fractions were denatured using SDS then MHC class II-α, MHC class I HC or calnexin immunoprecipitated.

US2 binds to MHC class II proteins

AdtetUS2 infected U373CIITAHis cells were treated with 35 μM ZL3VS, labeled with ^{35}S-methionine/cysteine for 5 minutes, then the label chased for 20 minutes. Sequential immunoprecipitations were performed by resuspending radio-labeled cells in 10 mM Hepes, pH 7.5, 10 mM $CaCl_2$ containing 1% digitonin, 1 mg/ml BSA and protease inhibitors and proteins immunoprecipitated as described (York *et al.*, *Cell* 77:525-535, 1994). The precipitated complexes were then dissociated and denatured in SDS lysis buffer, the SDS diluted ten-fold, and secondary immunoprecipitations performed.

Fig. 4A. US2 was immunoprecipitated using a rabbit polyclonal antibody, then proteins denatured with SDS, the SDS diluted and samples immunoprecipitated using anti-US2, anti-class II-α, anti-class II-ß, anti-Ii, anti-class I HC or anti- transferrin receptor (TfR) antibodies.

Fig. 4B. Extracts were immunoprecipitated using anti-class II-α, anti-class II-ß, anti-Ii, anti-class I HC or anti-transferrin receptor antibodies, samples denatured and reprecipitated using anti-US2 antibodies or antibodies directed to α, ß, Ii, HC, or TfR.

US2 inhibits presentation by MHCII on macrophages to CD4+ T cells

Fig. 5. CIITA-HIS16 cells were left uninfected or were infected with Adtet-trans, an adenovirus control without US2, or using AdUS2 and Adtet-trans, so that HCMV US2 was expressed in the cells, for 18 hours. The cells were washed and mixed with a CD4+ T cell clone that is specific for tuberculous antigen, and at the same time, with tuberculous antigen TbH9. The ability of the MHC class II antigen presenting CIITA-HIS16 cells to present TbH9 to the CD4+ T cells was measured. Specifically, the CD4+ T cells respond to antigen presented to them by producing interferon-gamma, and interferon-gamma levels were measured by enzyme-linked immunoassay (ELISA), and compared to known quantities of interferon-gamma. It was evident that the expression of US2 in the cells reduced the interferon-gamma response of the T cells, and therefore the MHC class II antigen presentation, by approximately 80%.

Fig. 6 is a schematic diagram of plasmid pΔE1sp1B and insert used to construct the Adtet vector of the present invention. The mapped sequence at the bottom of the figure was ligated into plasmid pΔE1sp1B (Microbix Biosystems, Inc., Toronto, Ontario, CA) at the HindIII and XhoI restriction endonuclease sites to produce the plasmid pΔE1sp1B-tet. The pΔE1sp1B-tet plasmid possesses a core HCMV promoter with flanking tet operator sequences, as well as an SV40 poly-adenylation site. The complex of tet operator sequences linked to the HCMV core promoter is called the tet responsive element. This element cannot effect transcription without an exogenous

transcription factor (ETF); the ETF can be expressed using a recombinant adenovirus (E1-). Furthermore, this transcription system is inactivated by tetracycline.

Fig. 7 is a comparison of the amino acid sequences of US2, US3, and US11, all of which bind to MHC class I. However, as shown in this specification, only US2 binds to MHC class II. By comparing all three related proteins, the sequences that are unique in US2 contain the regions responsible for, or involved in, binding to class II. Fig. 7 shows that residues 28-48 and 122-143 of US2 (underlined and marked with asterisk * in drawing) are less conserved regions between these proteins, and thus are likely to contribute to the binding domain(s) for MHC class II.

Soluble US2 blocks antigen presentation by T cell clones.

Fig. 8 is a graph showing that the soluble US2t-DL6 inhibits interferon-g secretion by T cells. This demonstrates that the soluble US2 protein blocks antigen presentation to the T cell clones.

SEQUENCE LISTINGS

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NO: 1 is the reverse complement of a cDNA sequence of the open reading frame that encodes US2.

SEQ ID NO: 2 is a cDNA sequence and corresponding amino acid sequence of the open reading frame that encodes US2.

SEQ ID NO: 3 is an amino acid sequence of US2.

SEQ ID NO: 4 is the nucleotide and amino acid sequence of the soluble US2 fusion US2t-DL6. This chimeric sequence contains the bee mellitin signal sequence (residues 1-63), a portion of the US2 cDNA (residues 64-471, corresponding to residues 58 to 465 of SEQ ID NO: 2), the sequence for a single glycine spacer (residues 472-474), a DL6 epitope (residues 475-531), and a stop codon (residues 532-534).

SEQ ID NO: 5 is the amino acid sequence of the soluble US2 fusion US2t-DL6. The bee mellitin signal sequence (corresponding to amino acid residues 1-21) is removed during translation to yield the final soluble US2 protein.

SEQ ID NOs: 6 and 7 are oligonucleotide primers that can be used to amplify the US2 cDNA sequence.

DETAILED DESCRIPTION

Abbreviations and Definitions

The following abbreviations and definitions are used herein:

CMV: Cytomegalovirus

HCMV: Human Cytomegalovirus

MHC: Major histocompatibility complex

Unless otherwise noted, technical terms are used according to conventional understanding. Definitions of common terms in molecular biology may be found in Benjamin Lewin, Genes V, Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the invention, the following definitions of terms are provided:

Animal: Living multicellular vertebrate organisms, a category that includes, for example, mammals and birds.

Binding domain (in a US2 molecule) for class I MHC: The region on the US2 protein that normally interacts (by binding, complexing or associating) with the class I MHC molecule. Specific amino acids of the US2 protein that are responsible for MHC chain binding can be determined through standard point-mutation and binding experiments (see, for instance, Fleury *et al.*, *Cell* 66:1037-1091, 1991; Olson *et al.*, *J. Biol. Chem.* 274:36905-36911, 1999). For instance, point mutations (*e.g.*, amino acid substitutions or short deletions) can be made in the US2 protein using well-known recombinant techniques, and the resultant US2 proteins assayed for MHC class I-linked US2 biological activity using methods described herein.

Binding domain (in a US2 molecule) for class II MHC: The region on the US2 protein that normally interacts (by binding, complexing or associating) with the class II MHC molecule. As discussed above for the MHC class I binding domain of US2, specific amino acids of the US2 protein that are responsible for MHC II binding can be determined through standard point-mutation and binding experiments. US2 activity assays may be those that examine MHC class II-linked US2 activity, as discussed herein. The binding domain within US2 for binding to class II MHC may share at least some residues with the US2 binding domain for class I MHC, and vice versa.

Binding domain (in a US2 molecule) for DM-α: The region on the US2 protein that normally interacts (by binding, complexing or associating) with the DM-α molecule. As discussed above for the MHC class I and MHC class II binding domain of US2, specific amino acids of the US2 protein that are responsible for DM-α binding can be determined through standard point-mutation and binding experiments. Examples of US2 activity assays are those that examine DM-α-linked US2 activity, as discussed herein. The binding domain within US2 for binding to DM-α may share at least some residues with the US2 binding domain for class I MHC and/or class II MHC, and vice versa.

CD4+ T cell mediated immunity: An immune response implemented by presentation of antigens to CD4+ T cells.

CD8+ T cell mediated immunity: An immune response implemented by presentation of antigens to CD8+ T cells.

DNA molecules that hybridize: DNA molecules and nucleotide sequences which are derived from the disclosed DNA molecules as described above may also be defined as DNA sequences which hybridize under stringent conditions to the DNA sequences disclosed, or fragments thereof.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing DNA used. Generally, the temperature of hybridization and the ionic strength (especially the Na^+ concentration) of the hybridization buffer will determine the stringency of hybridization. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989). chapters 9 and 11, herein incorporated by reference. By way of illustration only, a hybridization experiment may be performed by hybridization of a DNA molecule (for example, a variation of the US2 cDNA of SEQ ID NO: 2) to a target DNA molecule (for example, the US2 cDNA itself) which has been electrophoresed in an agarose gel and transferred to a nitrocellulose membrane by Southern blotting, a technique well known in the art and described in (Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989). Hybridization with a target probe labeled with [^{32}P]-dCTP is generally carried out in a solution of high ionic strength such as 6 x SSC at a temperature that is 20-25° C below the melting temperature, T_m, described below. For such Southern hybridization experiments where the target DNA molecule on the Southern blot contains 10 ng of DNA or more, hybridization is typically carried out for 6-8 hours using 1-2 ng/ml radio-labeled probe (of specific activity equal to 10^9 CPM/μg or greater). Following hybridization, the nitrocellulose filter is washed to remove background hybridization. The washing conditions should be as stringent as possible to remove background hybridization but to retain a specific hybridization signal. The term T_m represents the temperature above which, under the prevailing ionic conditions, the radio-labeled probe molecule will not hybridize to its target DNA molecule. The T_m of such a hybrid molecule may be estimated from the following equation:

$T_m = 81.5\ C - 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - 0.63(\%\ formamide) - (600/l)$
Where l = the length of the hybrid in base pairs.

This equation is valid for concentrations of Na^+ in the range of 0.01 M to 0.4 M, and it is less accurate for calculations of T_m in solutions of higher [Na^+]. The equation is also primarily valid for DNAs whose G+C content is in the range of 30% to 75%, and it applies to hybrids greater than 100 nucleotides in length (the behavior of oligonucleotide probes is described in detail in Ch. 11 of Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989).

The T_m of double-stranded DNA decreases by 1-1.5° C with every 1% decrease in homology. Therefore, for this given example, washing the filter in 0.3 x SSC at 59.4-64.4° C will produce a stringency of hybridization equivalent to 90%; that is, DNA molecules with more than 10% sequence variation relative to the target US2 cDNA will not hybridize. Alternatively, washing the hybridized filter in 0.3 x SSC at a temperature of 65.4-68.4° C will yield a hybridization stringency of 94%; that is, DNA molecules with more than 6% sequence variation relative to the target US2 cDNA molecule will not hybridize. The above example is given entirely by way of theoretical illustration. One skilled in the art will appreciate that other hybridization techniques may be utilized and that variations in experimental conditions will necessitate alternative calculations for stringency.

In certain embodiments of the present invention, stringent conditions may be defined as those under which DNA molecules with more than 25% sequence variation (also termed "mismatch") will not hybridize. In another embodiment, stringent conditions are those under which DNA molecules with more than 15% mismatch will not hybridize, and more stringent conditions are those under which DNA sequences with more than 10% mismatch will not hybridize. Very stringent conditions are those under which DNA sequences with more than 6% mismatch will not hybridize.

The degeneracy of the genetic code further widens the scope of the present invention as it enables major variations in the nucleotide sequence of a DNA molecule while maintaining the amino acid sequence of the encoded protein. Thus, the nucleotide sequence of the US2 cDNA could be changed without affecting the amino acid composition of the encoded protein or the characteristics of the protein. The genetic code and variations in nucleotide codons for particular amino acids is known. Based upon the degeneracy of the genetic code, variant DNA molecules may be derived from the cDNA molecules disclosed herein using standard DNA mutagenesis techniques as described above, or by synthesis of DNA sequences. DNA sequences that do not hybridize under stringent conditions to the cDNA sequences disclosed, by virtue of sequence variation based on the degeneracy of the genetic code, are herein also comprehended by this invention.

Injectable composition: A pharmaceutically acceptable fluid composition comprising at least one active ingredient, *e.g.* a US2 protein or variant, for instance a soluble variant. The active ingredient is usually dissolved or suspended in a physiologically acceptable carrier, and the composition can additionally comprise minor amounts of one or more non-toxic auxiliary substances, such as emulsifying agents, preservatives, and pH buffering agents and the like. Such injectable compositions that are useful for use with the fusion proteins of this invention are conventional; appropriate formulations are well known in the art.

Isolated: An "isolated" biological component (such as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, *i.e.*, other chromosomal and extra-chromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins that have been "isolated" thus include nucleic acids and proteins purified by standard

purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

Mammal: This term includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

Nucleic acid sequence (or polynucleotide): DNA or RNA molecule, and includes polynucleotides encoding full length proteins and/or fragments of such full length proteins which can function as a therapeutic agent.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

ORF (open reading frame): A series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences are usually translatable into a peptide.

Parenteral: Administered outside of the intestine, *e.g.*, not via the alimentary tract. Generally, parenteral formulations are those that will be administered through any possible mode except ingestion. This term especially refers to injections, whether administered intravenously, intrathecally, intramuscularly, intraperitoneally, or subcutaneously, and various surface applications including intranasal, intradermal, and topical application, for instance.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in this invention are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the US2 proteins and variants herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide preparation is one in which the peptide or protein is more enriched than the peptide or protein is in its natural environment within a cell. In certain embodiments, a preparation is purified such that the protein or peptide represents at least 50% of the total peptide or protein content of the preparation.

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques.

Sequence identity: the similarity between two nucleic acid sequences, or two amino acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar are the two sequences.

Methods of alignment of sequences for comparison are well-known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Bio.* 48:443, 1970; Pearson and Lipman, *Meth. Mol. Biol.* 24: 307-331, 1988; Higgins and Sharp, *Gene* 73:237-244, 1988; Higgins and Sharp, *CABIOS* 5:151-153, 1989; Corpet *et al.*, *Nucleic Acids Res.* 16:10881-90, 1988; Huang *et al., Computer Applications in BioSciences* 8:155-65,1992; and Pearson *et al.*, *Meth. Mol. Biol.* 24:307-31,1994. Altschul *et al.* (*Nat. Genet.,* 6: 119-29, 1994) presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403-410, 1990) is available from several sources, including the National Center for Biological Information (NBCI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at http://www.ncbi.nlm.nih.gov/BLAST/. A description of how to determine sequence identity using this program is available at http://www.ncbi.nlm.nih.gov/BLAST/blast_help.html.

Homologs of US2 are typically characterized by possession of at least 70% sequence identity counted over the full length alignment with the disclosed amino acid sequence of either the human, hamster, mouse, sheep, cow or other amino acid sequences using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Such homologous peptides may possess at least 75%, at least 80% or at least 90% or 95% sequence identity determined by this method. When less than the entire sequence is being compared for sequence identity, homologs will possess at least 75%, at least 85%, at least 90% or 95% sequence identity over short windows of 10-20 amino acids. Methods for determining sequence identity over such short windows are described at http://www.ncbi.nlm.nih.gov/BLAST/blast_FAQs.html. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is

entirely possible that strongly significant homologs or other variants could be obtained that fall outside of the ranges provided.

The present invention provides not only the peptide homologs that are described above, but also nucleic acid molecules that encode such homologs.

Therapeutic agent: Used in a generic sense, it includes treating agents, prophylactic agents, and replacement agents.

Therapeutically effective amount of a US2 protein: A quantity of US2 protein (or variant thereof) sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to inhibit an immune response, for instance an autoimmune response or an immune response to a graft, transplant, or gene therapy treatment. In general, this amount is sufficient to measurably inhibit a CD4+ mediated immune response.

An effective amount of US2 protein may be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the effective amount of US2 will be dependent on the specific protein applied, the subject being treated, the severity and type of the affliction, and the manner of administration of the fusion protein. For example, a therapeutically effective amount of US2 protein can vary from about 0.01 mg/kg body weight to about 1 g/kg body weight.

The US2 proteins and US2 protein variants disclosed in the present invention have equal application in medical and veterinary settings. Therefore, the general term "subject being treated" is understood to include all animals (e.g. humans, apes, dogs, cats, horses, and cows) that are or may experience an immune response that is susceptible to US2 protein-mediated inhibition.

Transformed: A transformed cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

US2 protein: A viral protein that is the translation product of the US2 gene of a cytomegalovirus, the DNA sequence of which is shown in SEQ ID NO: 2. The translated amino acid sequence is shown in SEQ ID NO: 3, and it is 180 amino acids in length after cleavage by signal peptidase, and 199 amino acids in length before cleavage of the signal sequence. The US2 protein includes a native cytomegalovirus US2 protein, which down-regulates expression of MHC I and MHC II, interferes with CD8+ and CD4+ T cell mediated immunity, and particularly interferes with the expression of the MHC class IIα subunit and the DM-α subunit to a greater extent than it interferes with expression of class IIβ or the invariant chain Ii. Alternatively, the US2 protein can be a translation product of a unique short open reading frame of the DNA sequence of SEQ ID NO: 2.

US2 binding domain: A region of the US2 protein that binds to a specific molecule, for instance a MHC class II, MHC class I, or DM-α polypeptide chain. US2 binding domains for each of

these proteins may share at least some residues, and are found in the region of about amino acid residue 20 to about amino acid residue 155 of SEQ ID NO: 3. In certain examples, US2 binding domains may be contained in about amino acid residues 28 to 48 and/or 122 to 143 of SEQ ID NO: 3, although all of these residues are not necessarily essential to binding.

Variant US2 polypeptides: A US2 protein (or peptide fragment) having one or more amino acid substitutions, one or more amino acid deletions, and/or one or more amino acid insertions, so long as the peptide retains the property of inhibiting CD4+ mediated immunity. Conservative amino acid substitutions may be made in at least 1 position, for example 2, 3, 4, 5 or even 10 positions, as long as the peptide retains inhibition of CD4+ mediated immunity. Such variant peptides or proteins include amino acid sequences with greater than about 60%, 70%, 80%, 90%, 95%, 98% or even 99% homology with the native US2 protein, or a subsequence thereof.

In certain examples, alterations of US2 made to create an US2 variant (*e.g.*, point mutations, deletions, etc.) will be entirely outside of the MHC II binding site of the US2 protein.

The term variant US2 polypeptide may also refer to a shortened fragment of the native US2 protein (SEQ ID NO: 3), so long as the fragment retains the property of inhibiting CD4+ mediated immunity. Appropriate fragments include, for instance, the entire soluble portion of the US2 protein (*i.e.*, residues about 1 through about residue 170), or shorter fragments, such as about residue 20 through about residue 155. Even shorter fragments may also be appropriate, for instance residues 20 through 165, through 160, through 155, or through 150 or thereabouts.

In some instances, it may be beneficial to remove the native US2 signal sequence in order to alter or influence the expression of the US2 variant. In certain of such embodiments, it may be beneficial to replace the native US2 signal sequence with a signal sequence from a different protein (*e.g.*, the bee mellitin signal sequence) or an artificially designed signal sequence, if entrance into the secretory pathway or outright secretion is desired. Signal sequences are well known.

In certain specific examples, soluble US2 proteins, or other US2 variants, are also beneficially expressed as fusion proteins with, for instance, a stabilizing or solubilizing protein domain joined to the US2 fragment or variant. Construction of soluble forms of proteins (*e.g.*, immunoglobulin fusions) that can bind to cells and inhibit immune responses is known (see, *e.g.*, Murphy *et al.*, *J. Exp. Med.*, 180:233-231, 1994; McKnight *et al.*, *J. Immuno.* 152:5220-5225, 1994; Linsley *et al.*, *Science* 257:792-795, 1992; Lenschow *et al.*, *Science* 257:789-792, 1992).

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in the host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art. The term "vector" includes viral vectors, such as adenoviruses, adeno-associated viruses, vaccinia, and retrovirus vectors.

EXAMPLE 1

Method of Inhibiting Immunity to Either a Viral Vector or a Transgene in Gene Therapy

In some embodiments, the present invention relates to a method of inhibiting immunity to either a viral vector or a transgene. This inhibition of immunity is useful for a number of purposes, including study of the immune response to vectors (or inhibition such a response), study of autoimmunity (or inhibition thereof), delivery of genes to cells. and in gene therapy. A general strategy for transferring genes into donor cells is disclosed in U.S. Patent No. 5,529,774, which is incorporated by reference. Generally, a gene encoding a protein having therapeutically desired effects is cloned into a viral expression vector, and that vector is then introduced into the target organism. The virus infects the cells, and produces the protein sequence in vivo, where it has its desired therapeutic effect. See, for example, Zabner *et al.*, *Cell* 75:207-216, 1993. However, one major limitation of this use of viral vectors for gene therapy is that the infected cells are recognized and killed by the CD4+ and CD8+ T lymphocytes, which eventually results in the loss of the virus and its beneficial therapeutic effect. In addition, CD4+ and CD8+ T lymphocyte responses are frequently directed to the transgene. Therefore, even if the virus vector can be made to be non-immunogenic, *e.g.* with "third generation" helper-dependent adenoviruses, T cell responses specific for foreign transgenes will frequently reduce or inhibit expression of that transgene.

The present invention offers a method by which the persistence of viral gene therapy vectors and expression of foreign transgenes might be improved, thereby enhancing the therapeutic value of the vectors. This method entails adding to the vector sequence a DNA sequence that encodes the US2 protein, or a US2 protein variant, or a substantially identical or substantially homologous protein, that inhibits CD4+ T cell recognition of infected cells. An added advantage of this method is that the US2 protein (and its variants and homologs) also effectively inhibits CD8+ T cell recognition of infected cells. Expression of this protein is designed to enhance the length of time the gene of interest is expressed, by inhibiting both CD4+ and CD8+ mediated immune responses that would otherwise eliminate the vector.

One embodiment of this invention is shown in Example 5, wherein the US2 gene has been isolated and inserted into AdtetUS2, a replication defective adenovirus vector that was constructed by coupling the US2 gene to a tetracycline inducible promoter. This vector was thereafter able to cause degradation of both MHC I and MHC II proteins as well as MHC II DM proteins (Fig. 2), and to inhibit both CD8+ and CD4+ mediated immune responses (Fig. 5). This example illustrates that a vector or vector element could be used to protect cells in which they reside from a CD4+ T cell mediated immune response, whether the gene therapy is carried out with that or another vector. Since it is the US2 product that causes the inhibition, this technique is generally applicable to the expression of US2 for improving the persistence of any virus in which such persistence may be desired.

As an alternative to adding the sequences encoding US2 or a homologous protein to the DNA of a virus, it is also possible to introduce such a gene into the somatic DNA of infected or uninfected

cells, by methods that are well known in the art (Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989). Alternatively, the sequence may be introduced into infected or uninfected cells by the use of a secondary virus, for example a virus that is not the virus that is expected to produce the immune response. A secondary virus may be introduced which carries the US2 coding sequence, prior to the introduction of a viral gene therapy vector. This possibility is well illustrated by Example 2 below, in which the gene for US2 is carried on an adenovirus vector, and is able to inhibit a T cell mediated immune response specific for CMV, with which the cells were co-infected. Hence it is clear that one vector carrying the gene for US2, or a homologous protein, could be used to infect a subject, and that a second vector, *e.g.* a virus carrying a therapeutic gene, could be used to infect the same subject. The first vector would then inhibit recognition by both CD4+ and CD8+ T cells.

A second use of the present invention is to prevent or inhibit organ rejection following transplantation of heterologous cells into a host. Rejection could be avoided or treated by supplying a therapeutically sufficient amount of US2 to the site of transplantation, either by exogenous administration or expression from cells in or near the organ that have been transformed to express US2.

Yet another use of the invention is in the treatment of autoimmune diseases, particularly autoimmune diseases in which therapeutic amounts of US2 are administered or provided to the patient. For example, a sequence that codes for US2 may be introduced into the cells of individuals suffering from autoimmune diseases, either by introducing the sequence into the somatic DNA or into a viral gene therapy vector, or alternatively, to introduce the protein US2 or its homologue into the subject's cells. The purpose of this is to limit display of both MHC I and II complexes, and particularly MHC II complexes, and thereby limit autoimmune responses and symptoms of the disease. This approach is believed to be useful in the treatment of many disorders believed to be at least in part mediated by T cell immunity, such as tissue and organ transplant rejection, diabetes, multiple sclerosis and arthritis. By reducing the recognition of cells of involved tissue by T cells, the symptoms of the disorder will be reduced. This therapy should also be of value in subjects at high risk for developing autoimmune disorders, even if the symptoms have not yet appeared.

In some of the foregoing examples, it may only be necessary to introduce the genetic or protein elements into certain cells or tissues. For example, in the case of diabetes, introducing them into only the pancreas should be sufficient. In the case of tissue or organ transplants, the elements may be introduced only into the tissue or organ being transplanted. However, in some instances, it may be more therapeutically effective and simple to treat all of the patients cells, or more broadly disseminate the vector, for example by intravascular administration.

Nonetheless, unlike immunosuppressive drugs that globally block immunity in all organs of the body, US2 could be delivered selectively to specific cells or organs and would more specifically block immune mediated damage to those specific cells.

Nucleic acid sequences encoding therapeutic agents that may be placed into the vector include, but are not limited to, nucleic acid sequences encoding tumor necrosis factor (TNF) genes, such as TNF-α; genes encoding interferons such as Interferon-α, Interferon-ß, and Interferon-γ; genes encoding

interleukins such as IL-1, IL-1ß, GMCSF, and interleukins 2 through 14; gene encoding neurotransmitters, neuromodulators, neurohormones, neurotropic factors, endothelial growth factors (EGF's), such as vascular endothelial growth and permeability factor (VEGPF); genes encoding fibroblast growth factors (FGF's), nerve growth factors (NGF's), choline acetyl transferase (CAT), the glial derived neurotrophic factor (GDNF); tyrosine hydroxylase; resistance to infections; the PMP-22 gene; the FMR-1 gene; neuroprotective genes; genes encoding inhibitory signals that reduce brain excitability; the ornithine transcarbamylase (OTC) gene; and negative selective markers or "suicide" genes, such as viral thymidine kinase genes, such as the Herpes Simplex Virus thymidine kinase gene, the cytomegalovirus virus thymidine kinase gene, and the varicella-zoster virus thymidine kinase gene, human alpha1-antitrypsin, the cystic fibrosis transporter, leptin, the leptin receptor, endothelial cell growth factors, inhibitors of hepatitis C virus, or cytosine deaminase.

The method of the present invention may be employed to treat diseases or disorders of a variety or organs, including the brain and central nervous system. Such diseases and disorders include, but are not limited to, ischemic strokes, angiogenesis, metabolic diseases of the brain, axonal injury, Alzheimer's disease, Parkinson's disease, Huntington's disease, central nervous system infections, mucopolysaccharidoses (MPS, types I-VII), lipidoses (such as, for example, Gaucher's disease), Lesch-Nyhan syndrome, X-linked ADL, metachromatic leukodystrophy, Krabbe's disease, Charcot-Marie-Tooth disease, Fragile X, stroke, epilepsies, Down's syndrome, phenylketonuria, degenerative disorders, mental disorders, and a variety of disorders that can be affected by introducing a new compound or modifying the levels of existing proteins in the brain or nervous system.

For example, a vector including a gene encoding an endothelial growth factor (such as vascular endothelial growth and permeability factor) or fibroblast growth factor (FGF), may be administered to a subject suffering from an ischemic stroke or with severely occluded arteries in the leg. In another example, a vector including a gene encoding nerve growth factor, or a gene encoding choline acetyl transferase, may be administered to a subject suffering from Alzheimer's disease. Other genes which may be contained in the vectors include, but are not limited to, genes encoding glial derived neurotrophic factor (GDNF), or tyrosine hydroxylasation for the treatment of Parkinson's disease; genes encoding resistance to central nervous system infections; the PMP-22 gene for treatment of Charcot-Marie-Tooth disease; the FMR-1 gene for treatment of Fragile X; neuroprotective genes to prevent further neuronal damage from stroke; and genes encoding inhibitory signals that reduce brain excitability for treatment of epilepsy.

The nucleic acid sequence encoding at least one therapeutic agent is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, the gene's native promoter, retroviral LTR promoter, or adenoviral promoters, such as the adenoviral major late promoter; the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; inducible promoters, such as the MMTV promoter; the metallothionein promoter; heat shock promoters; the albumin promoter; the histone promoter; the β-actin promoter; TK promoters; B19 parvovirus

promoters; and the ApoAI promoter. However the scope of the present invention is not limited to specific foreign genes or promoters.

The recombinant nucleic acid can be administered to the animal host by any method that allows the recombinant nucleic acid to reach the appropriate cells. These methods include injection, infusion, deposition, implantation, intra-arterial, oral ingestion or topical administration. Injections can be intramuscular, intravenous, intra-dermal, subcutaneous or intraperitoneal. Intravascular injections may use vectors that will transduce endothelial cells of blood vessels, such as those located in the brain, and produce the therapeutic agent in the endothelium. The recombinant nucleic acid can be delivered as part of a viral vector, such as a pox virus, recombinant vaccinia virus, a herpes virus, replication-deficient adenovirus strains or poliovirus, or as a non-infectious form such as naked DNA or liposome encapsulated DNA.

When administered as an intramuscular injection in humans, the dose range may be about 10^3 to 10^{13} infectious particles per injection, for example about 10^5 to 10^{11} infectious particles per injection, depending on the virus vector used. Single or multiple doses can be administered over time. With adenoviruses, one can inject as much as 10^{11} PFU/kg into monkeys; but for herpes viruses or pox viruses this can be 10^5 PFU total.

EXAMPLE 2

Adenoviral Vectors

In one embodiment, the vector used to express US2 (or a biologically active variant thereof, *e.g.*, a soluble US2 protein) and downregulate MHC-II is a viral vector. Viral vectors which may be employed include RNA viral vectors (such as retroviral vectors), and DNA virus vectors (such as adenoviral vectors, adeno-associated virus vectors, a herpesvirus, and vaccinia virus vectors). When an RNA virus vector is employed, in constructing the vector, the polynucleotide encoding the therapeutic agent is in the form of RNA. When a DNA virus vector is employed, the polynucleotide encoding the therapeutic agent is in the form of DNA.

Adenoviral vectors may include essentially the complete adenoviral genome (Shenk *et al.*, *Curr. Top. Microbiol. Immunol.* 111:1-39, 1984). Alternatively, the adenoviral vector may be a modified adenoviral vector in which a portion of the adenoviral genome has been deleted. The vector could be a so-called "first generation" Ad vector with deletions encompassing the E1 region or encompassing the E1 and E3 region of the Ad genome. Such vectors can be propagated on 293 cells that supply E1 gene products, as in Hitt *et al.* (*Meth. Mol. Gen.* 7:13-30, 1995). The vector could also be a so-called "second generation" adenovirus, which has deletions in the E1 region as well as in the E2 and/or E4 regions of the Ad genome. Such vectors can be propagated in 293 cells that have been transfected with E2 and/or E4 genes.

In one embodiment, the vector is a third generation adenovirus vector that includes an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA sequence encoding a therapeutic agent: and a promoter for expressing the DNA sequence encoding a therapeutic agent. The

vector could also be free of all Adenovirus ORFs and is propagated using 293-cre cells and a helper adenovirus that has loxP sequences flanking the DNA packaging sequences. Such third generation vectors do not engender anti AD immune responses, although they do engender anti-transgene responses. See Parks *et al.*, *Proc. Natl. Acad. Sci., USA* 93:13565 (1996) and Scheider *et al.*, *Nat. Gen.*, 18:180 (1998).

In another embodiment, the gene in the E2a region that encodes the 72 kilodalton (kDa) binding protein is mutated to produce a temperature sensitive protein that is active at 32° C, the temperature at which viral particles are produced, but is inactive at 37° C, the temperature of the animal or human host. This temperature sensitive mutant is described in Ensinger *et al.*, *J. Virol.*, 10:328-339, 1972; Van der Vliet *et al.*, *J. Virol.*, 15:348-354, 1975; and Friefeld *et al.*, *Virol.*, 124:380-389, 1983; Englehardt *et al.*, *Proc. Nat. Acad. Sci.*, 91: 6196-6200, 1994; Yang *et al.*, *Nat. Gen.*, 7:362-369, 1994.

Such a vector may be constructed according to standard techniques, using a shuttle plasmid which contains, beginning at the 5' end, an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a tripartite leader sequence, a multiple cloning site (which may be as herein described); a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The DNA segment serves as a substrate for homologous recombination with a modified or mutated adenovirus, and may encompass, for example, a segment of the adenovirus 5' genome no longer than from base 3329 to base 6246. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. A desired DNA sequence encoding a therapeutic agent may be inserted into the multiple cloning site of the plasmid.

The plasmid may be used to produce an adenoviral vector by homologous recombination with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral DNA sequences have been deleted. Homologous recombination may be effected through co-transfection of the plasmid vector and the modified adenovirus into a helper cell line, such as 293 cells, by $CaPO_4$ precipitation. The homologous recombination produces a recombinant adenoviral vector which includes DNA sequences derived from the shuttle plasmid between the Not I site and the homologous recombination fragment, and DNA derived from the E1 and E3 deleted adenovirus between the homologous recombination fragment and the 3' ITR.

Through such homologous recombination, a vector may be formed which includes an adenoviral 5' ITR, an adenoviral encapsidation signal; an E1a enhancer sequence; a promoter; a tripartite leader sequence; a DNA sequence encoding a therapeutic agent; a poly A signal; adenoviral DNA free of at least the majority of the E1 and E3 adenoviral DNA sequences; and an adenoviral 3' ITR. This vector may then be transfected into a helper cell line, such as the 293 helper cell line (ATCC No. CRL1573; available from Microbix Biosystems, Inc., Toronto, Ontario, CA), which will include the E1a and E1b DNA sequences, which are necessary for viral replication, to generate replication defective viral vector particles.

The vector may include a multiple cloning site to facilitate the insertion of DNA sequence(s) encoding therapeutic agent(s) into the cloning vector. In general, the multiple cloning site includes "rare" restriction enzyme sites, for example sites which are found in eukaryotic genes at a frequency of from about one in every 10,000 to about one in every 100,000 base pairs. An appropriate vector is thus formed by cutting the cloning vector by standard techniques at appropriate restriction sites in the multiple cloning site, and then ligating the DNA sequence encoding a therapeutic agent into the cloning vector.

In one embodiment, the adenovirus may be constructed by using a yeast artificial chromosome (or YAC) containing an adenoviral genome according to the method described in Ketner *et al.* (*Proc. Natl. Acad. Sci. USA*, 91:6186-6190, 1994), in conjunction with the teachings contained herein. In this embodiment, the adenovirus yeast artificial chromosome is produced by homologous recombination *in vivo* between adenoviral DNA and yeast artificial chromosome plasmid vectors carrying segments of the adenoviral left and right genomic termini. A DNA sequence encoding a therapeutic agent then may be cloned into the adenoviral DNA. The modified adenoviral genome then is excised from the adenovirus yeast artificial chromosome in order to be used to generate adenoviral vector particles as hereinabove described.

The adenoviral particles are administered in an amount effective to produce a therapeutic effect in a host. The particles may, for example, be administered IM, IP, IV or even intra-arterially (for example into the hepatic artery). In one embodiment, the adenoviral particles are administered intravascularly (for example through a catheter into an artery proximal to a target organ such as the brain) in an amount of at least 1×10^8 plaque forming units (pfu) per kg of body weight, and in general such amount does not exceed about 5×10^{11} pfu per kg of body weight, and generally is from about 1×10^9 pfu to about 1×10^{11} pfu per kg of body weight. The exact dosage of adenoviral particles to be administered is dependent upon a variety of factors, including the age, weight, and sex of the patient to be treated, and the nature and extent of the disease or disorder to be treated. The adenoviral particles may be administered as part of a preparation having a titer of adenoviral particles of at least 2×10^{10} pfu/ml, and in general not exceeding 2×10^{11} pfu/ml. The adenoviral particles may be administered in combination with a pharmaceutically acceptable carrier (which could increase adenovirus entry into cells or reduce immunity to virus particles) in a volume up to 10 ml. The pharmaceutically acceptable carrier may be, for example, a liquid carrier such as a saline solution, protamine sulfate (Elkins-Sinn, Inc., Cherry Hill, N.J.), or POLYBRENE™ (Sigma Chemical, St. Louis, MO).

EXAMPLE 3

Retroviral Vectors

In another embodiment, the viral vector is a retroviral vector. Examples of retroviral vectors which may be employed include, but are not limited to, Moloney Murine Leukemia Virus (MoMuLV), spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. The vector is generally a replication defective retrovirus particle.

Retroviral vectors are useful as agents to effect retroviral-mediated gene transfer into eukaryotic cells. Retroviral vectors are generally constructed such that the majority of sequences coding for the structural genes of the virus are deleted and replaced by the gene(s) of interest. Most often, certain viral structural genes (*i.e.*, gag, pol, and env) are removed from the retroviral backbone using genetic engineering techniques known in the art. This may include digestion with the appropriate restriction endonuclease or, in some instances, with Bal 31 exonuclease to generate fragments containing appropriate portions of the packaging signal.

New genes may be incorporated into proviral backbones in several general ways. In the most straightforward constructions, the structural genes of the retrovirus are replaced by a single gene which then is transcribed under the control of the viral regulatory sequences within the long terminal repeat (LTR). Retroviral vectors have also been constructed which can introduce more than one gene into target cells. Usually, in such vectors one gene is under the regulatory control of the viral LTR, while the second gene is expressed either off a spliced message or is under the regulation of its own, internal promoter. Alternatively, two genes may be expressed from a single promoter by the use of an Internal Ribosome Entry Site.

Efforts have been directed at minimizing the viral component of the viral backbone, largely in an effort to reduce the chance for recombination between the vector and the packaging-defective helper virus within packaging cells. A packaging-defective helper virus is necessary to provide the structural genes of a retrovirus, which have been deleted from the vector itself.

In one embodiment, the retroviral vector may be one of a series of vectors based on the N2 vector containing a series of deletions and substitutions to reduce to an absolute minimum the homology between the vector and packaging systems. These changes also reduce the likelihood that viral proteins will be expressed. In one such vector, LNL-XHC, the natural ATG start codon of gag is altered by site-directed mutagenesis to TAG, thereby eliminating unintended protein synthesis from that point. In MoMuLV, 5' to the authentic gag start, an open reading frame exists which permits expression of another glycosylated protein ($pPr80^{gag}$). Moloney murine sarcoma virus (MoMuSV) has alterations in this 5' region, including a frameshift and loss of glycosylation sites, which obviate potential expression of the amino terminus of $pPr80^{gag}$. The vector LNL6 incorporates both the altered ATG of LNL-XHC and the 5' portion of MoMuSV. The 5' structure of the LN vector series thus eliminates the possibility of expression of retroviral reading frames, with the subsequent production of viral antigens in genetically transduced target cells. In a final alteration to reduce overlap with packaging-defective helper virus, Miller has eliminated extra env sequences immediately preceding the 3' LTR in the LN vector (Miller *et al.*, *Biotechniques*, 7:980-990, 1989). Packaging-defective helper viruses for production of retroviral vectors are known in the art and examples thereof are described in Miller (*Hum. Gene Ther.*, 1:5-14, 1990).

In one embodiment, the retroviral vector may be a MoMuLV of the LN series of vectors, such as those herein mentioned above, and described further in Bender *et al. (J. Virol.* 61:1639-1646, 1987) and Miller *et al.* (*Biotechniques*, 7:980-990, 1989). Such vectors have a portion of the packaging signal

derived from a mouse sarcoma virus, and a mutated gag initiation codon. The term "mutated" as used herein means that the gag initiation codon has been deleted or altered such that the gag protein or fragments or truncations thereof, are not expressed.

In another embodiment, the retroviral vector may include up to or at least four cloning, or restriction enzyme recognition sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; for example, the restriction product has an average DNA size of at least 10,000 base pairs. Advantageous cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI. In a certain embodiment, the retroviral vector includes each of these cloning sites. When a retroviral vector including such cloning sites is employed, there may also be provided a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral vector. The shuttle cloning vector may also include at least one desired gene which is capable of being transferred from the shuttle cloning vector to the retroviral vector.

The shuttle cloning vector may be constructed from a basic "backbone" vector or fragment to which are ligated one or more linkers which include cloning or restriction enzyme recognition sites. Included in the cloning sites are the compatible or complementary cloning sites herein above described. Genes and/or promoters having ends corresponding to the restriction sites of the shuttle vector may be ligated into the shuttle vector through techniques known in the art. The shuttle cloning vector can be employed to amplify DNA sequences in prokaryotic systems, and may be prepared from plasmids generally used in prokaryotic systems and in particular in bacteria. Thus, for example, the shuttle cloning vector may be derived from plasmids such as pBR322 or pUC 18.

The vector includes one or more promoters. Suitable promoters that may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller *et al.* (*Biotechniques*, 7:980-990, 1989), or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and ß-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The vector then is employed to transduce a packaging cell line to form a producer cell line. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, PA12, T19-14X, VT-19-17-H2, GP+E-86, GP+envAm12, and DAN cell lines, as described in Miller (*Hum. Gene Ther.*, 1:5-14, 1990). The vector containing the nucleic acid sequence encoding the therapeutic agent may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation.

The producer cells generate retroviral vector particles, which are administered to a host, whereby the retroviral vector particles transduce cells of the host, and produce the therapeutic agent. The retroviral vector particles may be administered to the host in an amount effective to produce a therapeutic effect in the host. In general, the retroviral vector particles are administered in an amount of at least 1 x

10^8 colony forming units (cfu), and in general not exceeding 1 x 10^{10} cfu, and often about 10^9 cfu. The exact dosage of retroviral vector particles is dependent upon the factors already mentioned with respect to the adenoviral particles. The retroviral vector particles are administered as part of a preparation having a titer of retroviral vector particles of at least 1 x 10^7 cfu/ml and in general not exceeding 1 x 10^9 cfu/ml.

EXAMPLE 4

MHC II Expression Reduced in HCMV-Infected Cells

CD8+ and CD4+ T lymphocytes (T cells) are the principal immune defense against viral infections. These T cells recognize small peptides derived from viral proteins, and respond by rapidly killing virus infected cells, or orchestrating specific immune responses against the virus. Major histocompatibility (MHC) class I proteins are loaded with peptides in the endoplasmic reticulum (ER), where they are loaded with peptides that are subsequently presented to CD8+ T cells (York and Rock, *Ann. Rev. Immun.* 14:369-396, 1996). MHC class I proteins are ubiquitous, which enables CD8+ T cells to monitor the inside world of cells and detect viral proteins. By contrast, MHC class II proteins, which present to CD4+ T cells, are primarily expressed by professional antigen presenting cells, B cells, macrophages and dendritic cells, which take up extracellular proteins by endocytosis or phagocytosis and present on their surfaces peptides derived from the proteins. Without CD4+ T cells, which provide "help" in the form of costimulation, CD8+ T cell responses directed to viruses are usually weak, as in the case of AIDS.

Class II proteins are first synthesized in the endoplasmic reticulum (ER) as a complex of α and ß chains, bound to a third component, the invariant chain Ii, which protects the peptide-binding site (Wolf and Ploegh, *An. Rev. Cell & Dev. Biol.* 11:267-306, 1995; Cresswell, *Cell* 84:505-507, 1996). The α/ß/Ii complexes are transported to a lysosomal-like compartment known as the MHC class II compartment (MIIC), where peptide loading occurs. Another class II component, HLA-DM, binds to the class II complex and catalyzes exchange of a remnant of Ii for antigenic peptides produced by protease degradation of proteins in the MIIC (Denzin and Cresswell, *Cell* 82:155-165, 1995; Sloan *et al.*, *Nature* 375:802-806, 1995). From the MIIC, peptide-loaded class II complexes move to the cell surface for recognition by CD4+ T cell receptors.

To study the effects of HCMV infection on MHC class II molecules, a transformed glioblastoma cell line U373 was used, which is permissive for HCMV infection and can be induced to express MHC class II by IFN-γ treatment (Basta *et al.*, *J. Immun.* 138:1275-1280, 1987). There was a dramatic reduction in class II expression or stability within 12 hours of wild type (Wt) HCMV infection of IFN-γ treated U373 cells (Fig. 1A). When the cells were infected with an HCMV mutant unable to express a group of 12 genes, IRSI-1 and US2-US1 (Jones *et al.*, *J. Virol.* 69:4830-4841, 1995), there was no inhibition of class II expression. The US2-US11 region of the HCMV genome encodes at least 4 genes: US2, US3, US6, and US11, that independently block the MHC class I

pathway (reviewed in Johnson and Hill, *Curr. Top. Microbiol. Immun.* 232:149-177, 1998; and Ploegh, *Science* 280:248-253, 1998). To further characterize this effect, a U373-derived cell line, denoted U373-CIITAHis, was produced that constitutively expresses MHC class II proteins, by transfecting the cells with a class II transactivator gene, CIITA (Steimle *et al.*, *Science* 265:106-109, 1994). Infection of U373-CIITAHis cells with mutant viruses lacking various genes within the IRSI-US11 region (Jones *et al.*, *J. Virol.* 69:4830-4841, 1995) showed that mutants lacking both US2 and US3 were unable to cause this effect (Fig. 1B). The reduced expression of class II was not due to inhibition of IFN-mediated transcription of MHC class II genes, as described (Miller *et al.*, *J. Exp. Med.* 187:675-683, 1998), because mRNAs specific for the MHC class II α, ß and invariant chains were not reduced after 36 hours of HCMV infection (Fig 1C), yet diminished expression of class II proteins was observed after only 12 hours.

EXAMPLE 5

The HCMV US2 Protein Inhibits MHC II Expression

To assess whether US2, US3, or both were responsible for this inhibition, and whether US2 or US3 could be sufficient to downregulate class II proteins, we studied transfected cells expressing US2 or US3 and found that US2 but not US3 expression leads to diminished expression of class II. A replication-defective adenovirus (Ad) vector, AdtetUS2, expressing US2 was constructed by coupling the US2 gene to the tetracycline inducible promoter. US2 was expressed in U373 CIITAHis cells by co-infecting the cells with AdtetUS2 and a second virus, Adtet-trans, that expresses the tetracycline inducible element (Massie *et al.*, *Virol.* 72:2289-2296, 1998). In a pulse-chase experiment, there was obvious loss of expression of class II DR α/ß/Ii complexes in chase samples immunoprecipitated with a class II-α monoclonal antibody (MAb), and this was dependent on the quantity of US2 delivered (Fig. 2A). When class II complexes were first dissociated then immunoprecipitated with anti-α, anti-ß or anti-Ii chain antibodies, it was clear that the class II-α chain was rapidly lost and there was much less loss of ß and Ii (Fig. 2A). Some loss of expression of ß and Ii compared to control cells was observed, but this is likely related to the rapid loss of α chains, because ß chains are less stable in the absence of α (Dusseljee *et al.*, *J. Cell Science* 111: 2217-2226, 1998). Therefore, the loss of α appeared to be the primary event caused by US2. Expression of US2 caused degradation of the MHC class I HC, as expected (Wiertz *et al.*, *Nature* 384:432-438, 1996), but did not affect three cellular proteins: the transferrin receptor (TfR), and two ER-resident proteins, GRP94 and calnexin (Fig. 2A).

In cells expressing US2 and treated with specific inhibitors of the proteosome, lactacystin (Fenteany *et al.*, *Science* 268:726-731, 1995) or carbonylbenzyl-leucyl-leucyl-leucyl-vinylsulphone (ZL_3VS) (Bogyo *et al.*, *Proc. Natl. Acad. Sci., U S A* 94:6629-6634, 1997), class II α chains were stabilized (Fig. 3A). Thus, US2 induces degradation of class II α by the proteosome. In US2 expressing cells treated with a proteosome inhibitor, class I MHC was primarily present in a

deglycosylated form (Wiertz *et al.*, *Nature* 384:432-438, 1996) (Fig. 3A). By contrast, the class II α that accumulated under these conditions was primarily glycosylated (Fig. 3A). In order to determine if the class II α chain was translocated into the cytosol for degradation, US2-expressing cells were fractionated into a nuclear/ER fraction (1,000 X g), a dense vesicle/mitochondrial fraction (10,000 X g), a microsomal pellet (100,000 X g), and a cytosolic fraction (100,000 X g supernatant). Class II α was primarily found in dense membrane fractions, as was the case with calnexin an ER-resident protein, whereas class I HC was largely found in the cytosolic fraction in cells treated with ZL_3VS (Fig. 3B). Treatment of cells with tunicamycin to prevent addition of hydrophilic oligosaccharides did not increase the fraction of class II α found in the cytoplasm. These results demonstrate that US2 causes proteosome-mediated degradation of class II-α, but transfer of class II-α into the cytoplasm is either proteosome-dependent or does not occur.

EXAMPLE 6

US2 Binds to MHC II Proteins

The binding of US2 to class II-α, and other members of the class II complex, was measured by immunoprecipitating US2, denaturing the precipitated proteins, then reprecipitating with anti-α, anti-ß or anti-Ii antibodies. In cells labeled for 5 minutes, then the label chased for 20 minutes, there was obvious association of US2 with the α chain, and with the class I HC (Fig. 4A). However, there was also coprecipitation of ß and Ii with US2, less than that observed with α. Similarly, anti-α, anti-ß, and anti-Ii antibodies precipitated US2 (Fig. 4B). Therefore, it appears that US2 binds to the entire class II complex, although there also appears to be some association between US2 and free class II-α chains. It appears that most of the α chains labeled under these conditions were rapidly assembled into a/ß/Ii complexes. Given these results, it is significant that US2 specifically mediates degradation of class II-α.

EXAMPLE 7

US2 Also Degrades DM-α

The observation that US2 can cause degradation of both MHC class II α chains and class I HC, was surprising, because these molecules are not extensively homologous (Fig. 1D). Related to this, US2 can bind to and degrade MHC class I HLA-A and HLA-B, but not HLA-C and HLA-G that show extensive sequence homology (Fig. 1D) (Schust *et al.*, *J. Exp. Med.* 188:497-503, 1998). Another member of the MHC class II pathway, HLA-DM, binds to the class II α/ß complex in MIIC compartments, catalyzing the loading of peptides. HLA-DM proteins show some limited homology to the classical MHC class II DR proteins (Fig. 1D). Thus, it was of interest to determine whether US2 could cause degradation of DM proteins. DM-α was rapidly degraded in US2 expressing cells, whereas DM-ß was not degraded more rapidly than in control cells (Fig. 2B). Therefore, US2 causes degradation of DM-α and class II DR-α, as well as class I HC proteins. By destroying both MHC

class II proteins and, in addition, HLA-DM proteins, the effects of HCMV US2 on MHC class II presentation to CD4+ T cells are enhanced.

EXAMPLE 8

US2 Expression in Moncocytes/Macrophages Blocks Recognition of the Cells by CD4+ T cells

Monocyte/macrophages play a pivotal role in the lifecycle of HCMV and the cells express MHC class II proteins acting as professional antigen presenting cells. In this example, it is shown that US2 expression in macrophages blocks the MHC class II pathway, and presentation of antigens to CD4+ T cells. When infecting cultured macrophages with HCMV, only 10-15% of the macrophages exhibited evidence of infection. As an alternative, US2 was introduced into macrophages by using AdtetUS2. The macrophages were incubated with tetanus toxin, an antigen that can be processed and presented by the macrophages, then the macrophages were irradiated and mixed with tetanus toxin-specific CD4+ T cells. Proliferation of the CD4+ T cells (in response to the tetanus toxin) was measured. US2 expression in the macrophages markedly reduced presentation of the tetanus toxin to the CD4+ T cells, in a dose-dependent manner, whereas infection with a control virus, AddlE1, had no effect (Fig. 5). Therefore, US2 effectively inhibits antigen presentation by the class II pathway in macrophages.

HCMV US2 is the first viral inhibitor of the MHC class II pathway to be described, and is the only reported specific inhibitor of the MHC class II antigen presentation pathway. By causing degradation of class II DR and DM complexes, US2 should confer resistance to CD4+ cytotoxic T lymphocytes (CTL). Moreover, since CD4+ T cells can provide costimulation for CD8+ T cells by binding to the same antigen presenting cell (Schoenberger *et al.*, *Nature* 393:480-483, 1998), US2 may indirectly reduce CD8+ CTL responses. The downregulation of both class I and class II pathways in professional antigen presenting cells would allow global reductions in cellular and humoral immunity, and sustained replication in antigen presenting cells. Hence US2 can be used to block inappropriate immune responses in autoimmunity, transplantation or against gene therapy vectors.

EXAMPLE 9

Construction of Adtet US2 and Expression of US2 in Cells

The US2 gene (the US2 open reading frame of SEQ ID NO: 2) was first described by Weston and Barrell (*J. Mol. Biol.* 192:177, 1986), who sequenced the unique short component of the human cytomegalovirus genome. The entire US2 gene was subcloned by using PCR primers or oligonucleotides: US2 forward (SEQ ID NO: 6: 5' CGC GGA TCC ATG AAC AAT CTC TGG AAA GCC TGG 3') and US2 reverse (SEQ ID NO: 7: CGT GAA TTC GAC ATG ACA CAC GTA ATG GGT ACT). The PCR reaction was performed using HCMV DNA purified from HCMV-infected human fibroblasts. The 600-nucleotide PCR product generated with these two

oligonucleotides was cut with EcoRI and Xbal and inserted between the EcoRI and Xbal sites of pΔE1sp1B-tet (Fig. 6). pΔE1sp1B-tet was produced previously by altering pΔE1sp1B (Microbix Biosystems, Inc., Toronto, Ontario, CA; Brett *et al.*, *Proc. Natl. Acad. Sci. USA* 91:8802-8806, 1994), by inserting a tetracycline-responsive promoter cassette between the HindIII and Xhol sites (see map in Fig. 6). The tetracycline-responsive promoter consists of a tetracycline operator sequence coupled to a HCMV core promoter element (see map in Fig. 6).

pΔE1sp1B-tet was cotransfected into 293 cells along with a second plasmid pJM17 (see, Hitt *et al.*, *Meth. Mol. Gen.* 7:13-30, 1995), a plasmid that contains the remainder of the Ad genome. Together these two plasmids recombined to give rise to an E1-, replication-defective Ad vector, denoted AdtetUS2. The 293 cells supply Ad E1 proteins and allow replication of E1-Ad vectors. However, when cells other than 293 cells (that do not express E1) are infected, E1-Ad viruses do not replicate, but can express heterologous proteins. The tetracycline-repressible promoter in AdtetUS2 does not cause expression on the US2 gene unless cells are also coinfected with a second Ad vector, Adtet-trans. Adtet-trans expresses a transactivator protein, composed of the tetracycline repressor fused to the herpes simplex virus VP16 transactivating domain (Gossen and Bujard, *Proc. Natl. Acad. Sci., USA* 89:5547-5551, 1992). This tet transactivator protein binds to tet operator sequences in the promoter of AdtetUS2 and activates expression of the US2 gene. Therefore, to obtain expression of US2, cells are coinfected with AdtetUS2 and Adtet-trans.

Adenovirus vectors such as Adtet US2 or others in which the US2 was coupled to other promoters could be used to express US2 as well as other therapeutic transgenes. Given that US2 will reduce MHC class II antigen presentation of adenovirus and transgene antigens to CD4+ T cells, US2 would be expected to sustain long term expression by these adenovirus vectors. Both CD4+ and CD8+ T cell responses reduce and shorten expression of transgenes by Ad vectors.

Other adenoviral vectors could also be used to express the US2 in conjunction with other inhibitors of the immune system. Such adenoviral vectors could include but not be limited to Ad vectors, expressing US2 as well as ICP47, the human CMV UL18 gene product that blocks NK cells, HCMV USII, or pox virus proteins that inhibit inflammation. Adenoviral vectors could also include third-generation or helper-dependent Ad vectors, as described in Parks *et al.*, *Proc. Natl. Acad. Sci. USA* 93:13565-13570, 1996, and vectors expressing a variety of therapeutic transgenes, such as the cystic fibrosis transporter, human alpha-1-antitrypsin, leptin, citrullinaemia, and others.

EXAMPLE 10

Use of US2 to Treat Organ Rejection or Autoimmune Diseases

Expression of US2 could be used to treat rejection following organ transplantation. The liver would be removed from the donor, and AdtetUS2 introduced into the organ, for example by perfusing the portal vein with 10^{12} pfu of AdtetUS2 (as well as 5-fold less Adtet-trans). Allogenic transplantation of the liver would then be performed into a heterologous host. The recipient would be immunosuppressed with cyclosporine for a period of time (for example, a few days to two weeks).

After that time, expression of US2 (and perhaps ICP47) would have had time to reduce surface MHC class I and class II expression, to inhibit the allogenic T lymphocyte response directed to the transplanted organ (Drazan *et al.*, *Transpl.* 59:670-673, 1995; and Olthoff *et al.*, *Transp. Proc.* 29:1030-1031, 1997).

Alternatively, hepatocytes prepared from liver biopsies or livers removed from donors by collagenase treatment could be infected with AdUS2 vectors then the hepatocytes introduced into the portal vein, or into the spleen of a recipient. Such reintroduced hepatocytes can repair damaged livers in recipients in animal models. In humans, the hepatocytes are frequently destroyed by immune responses, but US2 could blunt these immune responses. Alternatively, in cases of heart transplantation, the entire heart could be transduced with the viral vector (Qin *et al.*, *Hum. Gene Ther.* 8:1365-1374, 1997).

In the treatment of pancreatic disorders, isolated pancreatic islets could be targets for gene therapy, as in Csete *et al.* (*Transplantation* 59:263-268, 1995). The pancreatic islet cells could be infected with AdtetUS2/Adtet-trans or other Ad vectors carrying immunosuppressive genes. The cells could then be reintroduced into an allogenic recipient to restore partial pancreatic function (as in Platt *et al.*, *Nat. Med.* 3:26-27, 1997; and Gunsulas *et al.*, *Nat. Med.* 3:48-53, 1997).

EXAMPLE 11

Conditions Associated Primarily with $CD4^+$ T Cell Immunity, or MHC II

$CD4^+$ mediated immunity (via MHC II presentation) is associated with the immune response provoked by gene therapy vectors, as well as transplantation and autoimmune disorders.

EXAMPLE 12

Soluble US2 Molecules

As discussed above, US2, a membrane protein, can bind to MHC class II proteins and inhibit the class II antigen presentation pathway, by causing degradation of the class II proteins. This occurs in the endoplasmic reticulum of HCMV infected or US2-transduced cells.

It is advantageous in certain circumstances to formulate the inhibitory US2 in a soluble (non-membrane bound) form. Soluble US2 can be constructed using standard genetic engineering techniques. Additionally, it is sometimes advantageous to express the soluble US2 as a fusion protein with, for instance, a stabilizing or solubilizing protein domain functionally joined to the US2 fragment or variant. The chimeric domain can be, for instance, an immunoglobulin domain (see, *e.g.*, Murphy *et al.*, *J. Exp. Med.*, 180:233-231, 1994; McKnight *et al.*, *J. Immuno.* 152:5220-5225, 1994; Linsley *et al.*, *Science* 257:792-795, 1992; Lenschow *et al.*, *Science* 257:789-792, 1992). The construction of fusion proteins, and more particularly soluble fusion proteins, is well known to one of ordinary skill in the art.

Merely by way of example, below is a discussion of the construction of one useful soluble US2 fragment, which includes the majority of the soluble extracellular portion of the US2 protein but does not include the generally C-terminal transmembrane domain and intracellular domain.

Construction of recombinant baculovirus.

In order to express a soluble form of US2, a recombinant baculovirus vector was used to infect the cells and cause high level expression of this US2 recombinant protein. The bBM-US2-DL6 baculovirus was made by using the polymerase chain reaction to produce a DNA sequence encoding the US2 protein fused to an antibody epitope (the DL6 epitope of herpes simplex virus glycoprotein D) at the extreme C-terminus. This epitope was added merely to facilitate study of this soluble protein, and is not required for the functionality of the soluble US2.

In order to cause secretion of the US2 protein into the medium, a signal sequence can be included in the construct. In this example, the bee mellitin signal sequence was added at the N-terminus of the recombinant protein, replacing the native (US2) signal sequence. The resultant amplified PCR product (containing bee mellitin, US2, and DL6 sequences) was inserted into a shuttle vector pFASTBAC1 (GIBCO, Gaithersburg, MD) at BamHI and EcoRI restriction sites.

To create a baculovirus expressing the recombinant US2 protein, pFASTBAC1 was recombined with a baculovirus genome cloned into a bacterial artificial chromosome (BAC) present in *E. coli* cells strain DH10BAC (GIBCO, Gaithersburg, MD). Recombination between the soluble US2 construct and the BAC produced a BAC containing the US2 sequences. This BAC was extracted from the DH10BAC bacteria and transfected into Tn5 insect cells to obtain a baculovirus, bBM-US2-DL6, capable of expressing recombinant US2 (US2t-DL6). The expressed and secreted protein (SEQ ID NO: 5) contains the following amino acid sequences: US2 amino acids 20-155 (from SEQ ID NO: 3), followed by a single glycine residue and DL6 epitope (amino acid residues 159-177, SEQ ID NO: 5). The bee mellitin signal sequence (amino acid residues 1-21, SEQ ID NO: 5) is removed during the synthesis of the protein.

Purification of recombinant protein from insect cells.

Large scale preparations of Tn5 insect cells were infected with baculovirus bBM-US2t-DL6 using 0.5-1.0 plaque forming units per cell. After 100 hours of infection, the cell culture supernatants were harvested. The cells and debris were removed by low speed centrifugation and the supernatants were passed onto a column containing the DL6 monoclonal antibody (fixed to Amino-link, Pierce Chemical Co.) The column was washed with 5 column volumes of phosphate-buffered saline then the recombinant US2t-DL6 was eluted by passing several volumes of 0.1 M ethanolamine, pH 11.0, over the column. The protein preparations were immediately neutralized to pH 7.4 with 3 M sodium acetate, pH 5.0 and dialyzed extensively against phosphate buffered saline, pH 7.2.

Inhibition of antigen presentation to CD4+ T cells by US2t-DL6.

To test the ability of US2t-DL6 to inhibit antigen presentation, Epstein-Barr Virus-transformed lymphoblastoid cells or LCLs (which express relatively high levels of MHC class II proteins and can present exogenous antigen to MHC class II-restricted, CD4+ T cell clones) were used. U373 glioma cells that express little or no MHC class II protein and cannot present antigens to CD4+ T cells were used as a negative control. These cells were incubated with various concentrations of the US2t-DL6 protein for 4 hours. Following this incubation the cells were incubated with US2t-DL6 and an antigenic protein, Mtb39, derived from *Mycobacterium tuberculosis*. Mtb39 can be recognized by Mtb39-specific, CD4+ T cell clones, after the Mtb39 proteins is taken by endocytosis, digested into peptides and presented on the appropriate class II proteins present on the antigen presenting cells, in this case, LCL or U373 cells. Simultaneously, the cells were incubated with CD4+ T cells clones specific for Mtb39. This incubation was for 18 hours and subsequently cell culture supernatants were harvested and analyzed for the presence of the cytokine interferon-γ which is released by the T cells after the cells recognize antigen (Mtb39) presented by the appropriate MHC class II proteins. The US2t-DL6 protein was able to block antigen presentation to the T cell clones as shown by the inhibition of the interferon-γ secretion by the T cells (Fig. 8). Half-maximal inhibition of the T cells was observed with 4-11 μg/ml of the US2t-DL6 protein. We conclude that this soluble form of US2 can bind to class II proteins on the surfaces of antigen presenting cells (LCL) and can effectively block recognition of these class II proteins by CD4+ T cells.

Example 13

Clinical us of soluble US2 molecules

As shown in Example 12, a soluble, truncated form of US2 can effectively inhibit recognition of antigen presenting cells by CD4+ T cells in an in vitro assay. There is precedent for the use of soluble chimeric proteins derived from native cellular proteins to inhibit CD8+ and CD4+ T cells responses *in vivo*. Previously, a soluble form of CTLA4 has been used to block various types of immunity in experimental animals and in human patients (Lenschow *et al.*, *Science* 257:789-792, 1992). CTLA-4 is a membrane protein found on T lymphocytes, and binds B7-1 and B7-2 costimulatory molecules on antigen presenting cells (Schwartz, *Cell* 71:1065-1071, 1992). CTLA-4-IgG is a fusion protein that contains a soluble, truncated form of CTLA-4 fused to the immunoglobulin G (IgG) constant domain. The CTLA-4-IgG protein is able to block the interaction between the CTLA-4 molecules on T cells and B7-1 or B7-2 on antigen presenting cells and, by doing so can effectively inhibit T cell responses. CTLA-4-IgG can be injected into animals or humans and blocks T cells responses *in vivo*. This soluble form of CTLA-4 acts as a immunosuppressive agent, and can be used to reduce immunity directed to transplanted tissues (Lenschow *et al.*, *Science* 257:789-792, 1992), to gene therapy vectors (Jooss *et al.*, *Gene Therapy* 5:309-19, 1998), or during other inappropriate T cell mediate immune responses, for instance in autoimmunity (*e.g.*, diabetes).

This invention encompasses soluble, truncated forms of US2, including the one described herein and others where the US2 MHC binding domain is fused to the immunoglobulin G (IgG) constant domain or other domains, that can be used to block inappropriate immune responses *in vivo*. US2-based proteins can be injected into the bloodstream or directly into specific tissues. The presence of soluble forms of US2 in the body or in specific tissues may block recognition of antigen presenting cells by CD8+ or CD4+ T lymphocytes, as indicated by Fig. 8. This inhibition could be useful to block inappropriate T cell immunity that occurs in autoimmunity (*e.g.*, diabetes, arthritis) or during tissue or cell transplantation or to inhibit T cell responses directed to foreign transgenes or virus vectors in gene therapy.

The US2 proteins of this invention may be administered to humans, or other animals on whose cells they are effective, in various manners such as topically, orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, intrathecally, and subcutaneously. The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (*e.g.*, the subject, the disease, and the disease state involved, and whether the treatment is prophylactic or post-infection). Treatment may involve daily or multi-daily doses of US2 protein(s) over a period of a few days to months, or even years.

US2 proteins and US2 variants, including those specifically described herein, are particularly useful in the inhibition of inappropriate or detrimentally extreme immune responses, such as those that may occur in the course of an autoimmune disease, or be associated with organ or tissue transplantation, or gene therapy.

In view of the many possible embodiments to which the principles of our invention may be applied, it should be recognized that the illustrated embodiment is only an example of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method for inhibiting recognition of cellular tissues by both CD8+ and CD4+ T cells, comprising introducing into a mammalian cell or tissue an isolated nucleotide sequence encoding HCMV US2, or a variant thereof which maintains the activity of US2, and wherein the binding domain recognizes:

(a) MHC class I heavy chains and MHC class II α chains;

(b) DM-α chains; or

(c) MHC class I heavy chains, MHC class II α chains, and DM-α chains.

2. The method of claim 1, wherein the binding domain of US2 is a native binding domain.

3. The method of claim 1, wherein the binding domain of US2 is a binding domain that has not been mutated to recognize MHC II molecules.

4. The method of claim 1, wherein the nucleotide sequence is introduced through the use of a viral vector.

5. The method of claim 1, wherein the US2 variant is a soluble US2 variant.

6 The method of claim 5, wherein the soluble US2 variant comprises a sequence as shown in amino acid residues 28 through 143 of SEQ ID NO: 3.

7 The method of claim 6, wherein the soluble US2 variant comprises a sequence as shown in amino acid residues 20 through 155 of SEQ ID NO: 3.

8. A recombinant expression vector comprising a promoter operably linked to a nucleotide sequence encoding an US2 protein, or a variant thereof, wherein a binding domain of the US2 has not been mutated to recognize MHC II molecules, which expression vector, when present in a mammalian cell, inhibits the ability of the cell to present antigens associated with MHC class II proteins to T cells.

9. A method of improving the persistence of a virus by introducing into the viral genome an isolated nucleotide sequence encoding a US2 protein, or a variant thereof which maintains the activity of US2. that has a binding domain that has not been mutated to increase recognition of MHC II molecules.

10. A vector able to suppress MHC I and MHC II mediated immunity, which comprises an isolated nucleotide sequence that encodes a protein having the activity of native US2.

11. The vector of claim 10, wherein the vector is a viral vector.

12. The vector of claim 11, wherein the vector is an adenoviral vector.

13. The vector of claim 11, wherein the vector is a retroviral vector.

14. The vector of claim 10, wherein the protein having the activity of native US2 is a soluble US2 protein.

15. A method of preventing or treating an autoimmune disease, comprising administering to a subject a therapeutically effective amount of US2 of claim 10.

16. The method of claim 15, wherein the autoimmune disease is mediated by MHC II molecules.

17. A method of improving gene therapy, comprising administering to a subject the vector of claim 10.

18. The method of claim 17, wherein the vector also comprises a nucleotide sequence that codes for a therapeutic product.

19. A method for inhibiting recognition of cells or tissues by both CD8+ and CD4+ T cells, comprising introducing into a mammal a purified US2 protein, or a therapeutically effective fragment thereof.

20. The method of claim 19, wherein the therapeutically effective US2 fragment is a soluble US2 fragment.

21. The method of claim 19, wherein the purified protein is exogenously supplied.

22. The method of claim 19, wherein the purified protein is expressed from a recombinant cell.

23. A purified soluble protein having US2 protein biological activity, and comprising an amino acid sequence selected from the group consisting of:

(a) residues 28 through 143 shown in SEQ ID NO: 3;

(b) amino acids sequences that differ from those specified in (a) by one or more conservative amino acid substitutions; and

(c) amino acid sequences having at least 70% sequence identity to the sequences specified in (a) or (b).

24. An isolated nucleic acid molecule encoding a protein according to claim 23.

25. A soluble protein according to claim 23, comprising the amino acid sequence shown in SEQ ID NO: 5.

26. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence according to claim 24.

27. A cell transformed with a recombinant nucleic acid molecule according to claim 26.

28. An isolated nucleic acid molecule encoding a soluble US2 protein.

29. A method for inhibiting a CD4+ mediated immune response, comprising introducing into a mammal a purified US2 protein, or a therapeutically effective fragment thereof.

30. The method of claim 29, wherein the therapeutically effective US2 fragment is a soluble US2 fragment.

31. A method for inhibiting an immune response in a subject, comprising administering to the subject an amount of a US2 protein, sufficient to inhibit the immune response.

32. The method according to claim 31, wherein the protein is administered in the form of a pharmaceutical composition.

33. The method according to claim 31, wherein the immune response is a CD4+ mediated immune response.

34. The method according to claim 31, wherein the immune response is selected from the group consisting of autoimmune responses, transplant immune responses, and gene therapy immune responses.

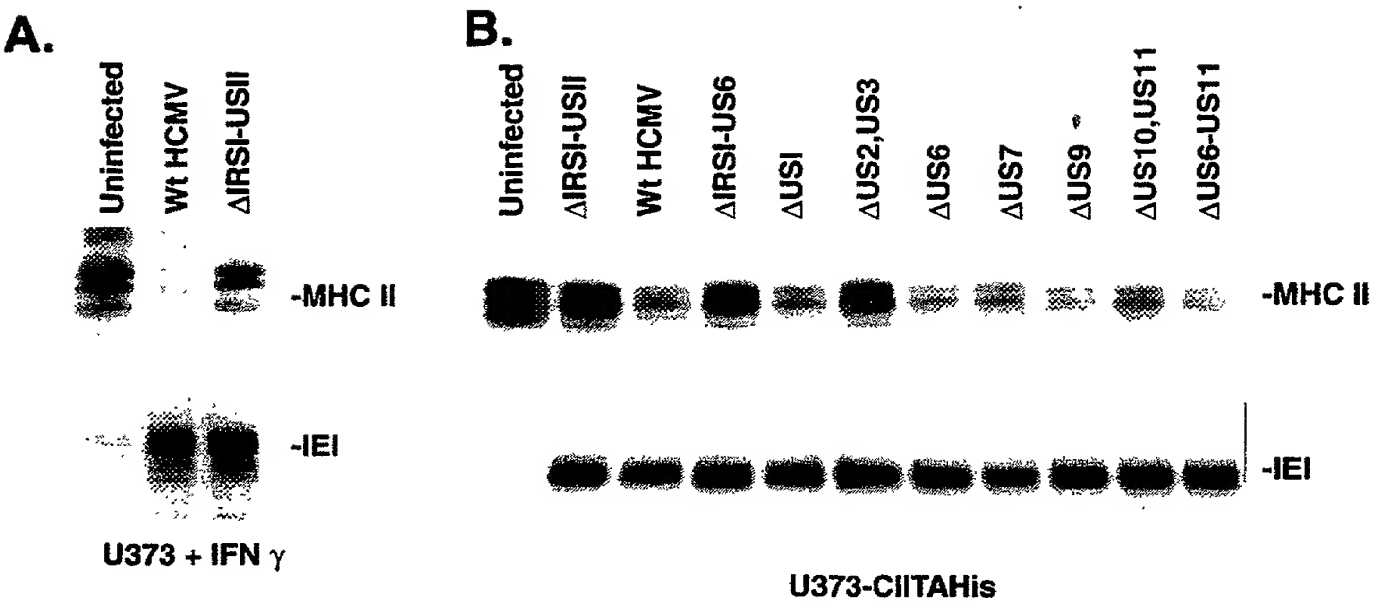
35. The method of claim 31, wherein the US2 protein is soluble.

36. The method of claim 35, wherein the soluble US2 protein is the soluble US2 protein of claim 23.

37. A composition comprising the protein according to claim 23.

38. A pharmaceutical composition comprising the protein according to claim 23 and a pharmaceutically acceptable carrier.

Fig 1.


A.
Uninfected
Wt HCMV
ΔIRSI-USII
-MHC II
-IEI
U373 + IFN γ
B.
Uninfected
ΔIRSI-USII
Wt HCMV
ΔIRSI-US6
ΔUSI
ΔUS2,US3
ΔUS6
ΔUS7
ΔUS9
ΔUS10,US11
ΔUS6-US11
-MHC II
-IEI
U373-CIITAHis
C.
Uninfected
Wt HCMV
ΔIRSI-USII
DRα
CIITA
DRβ
HCMV-gB
Ii
β-actin
D.
HLA-G
81%
HLA-A
HLA-C
80%
Ig-λ
22%
18%
28%
27%
24%
28%
DRβ
26%
DRα
31%
DMα
25%
DMβ

09/890806
PCT/US00/02740

Fig 2.

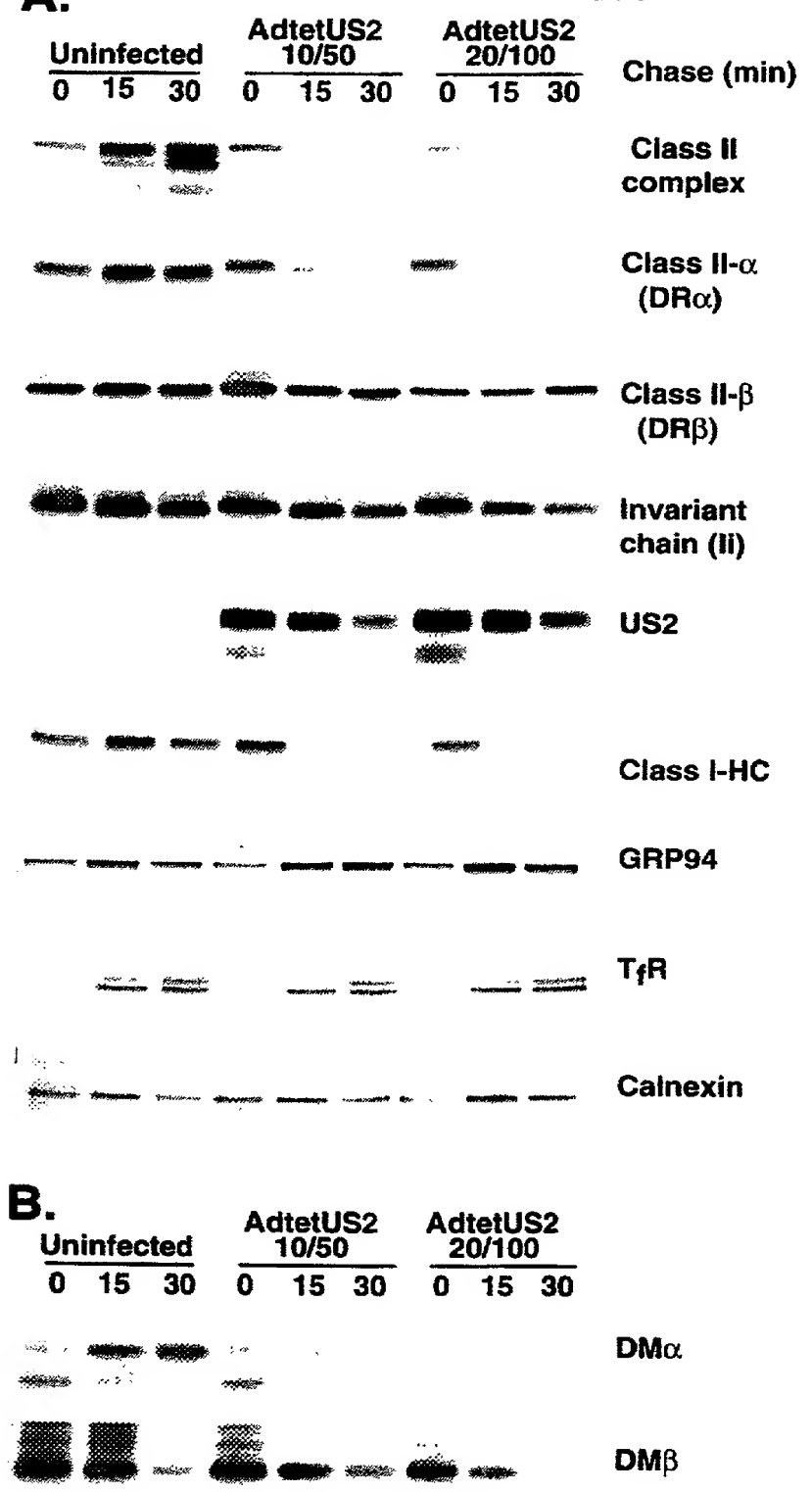


Fig 3.

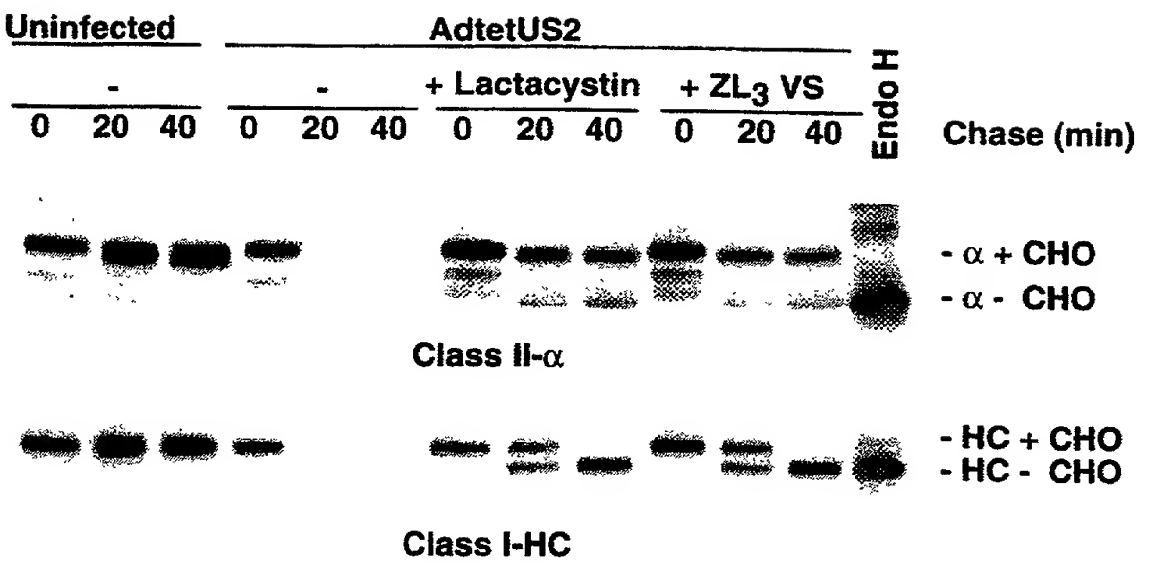
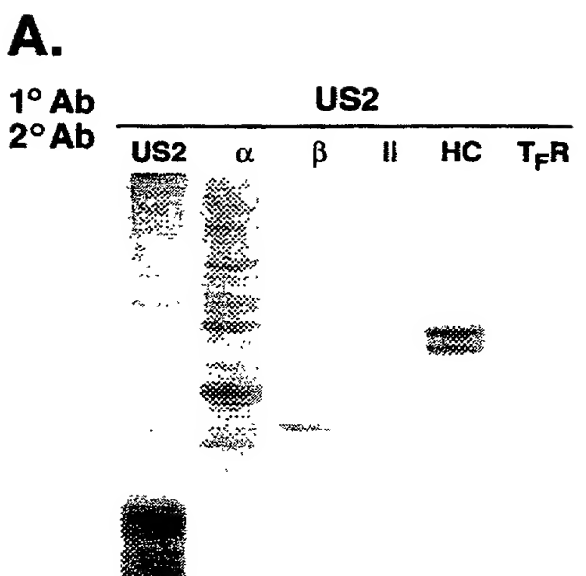

A.
Uninfected
AdtetUS2
-
-
+ Lactacystin
+ ZL3 VS
Endo H
0 20 40 0 20 40 0 20 40 0 20 40
Chase (min)
- α + CHO
- α - CHO
Class II-α
- HC + CHO
- HC - CHO
Class I-HC
B.
1000g pellet
10,000g pellet
100,000g pellet
100,000g supernatant
10 20 40 10 20 40 10 20 40 10 20 40
Chase (min)
- α + CHO
- α - CHO
Class II-α
- HC + CHO
- HC - CHO
Class I-HC
Calnexin

Fig.4



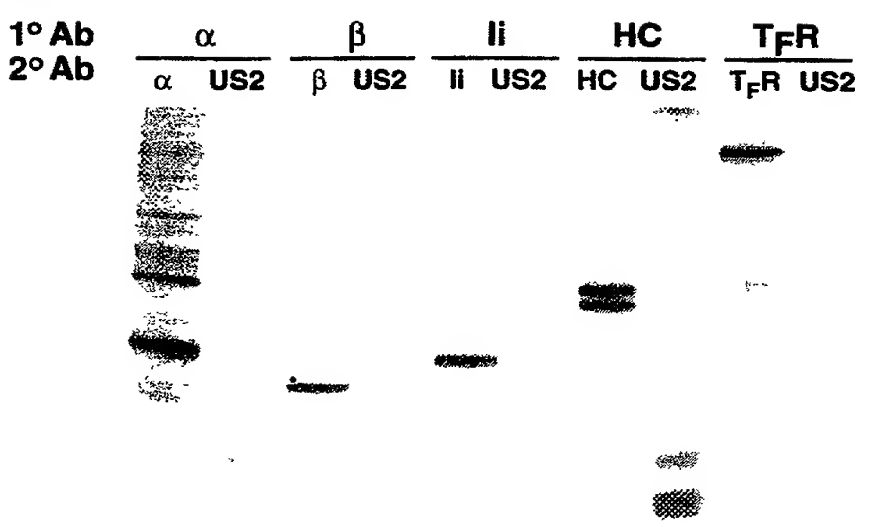


Fig. 5

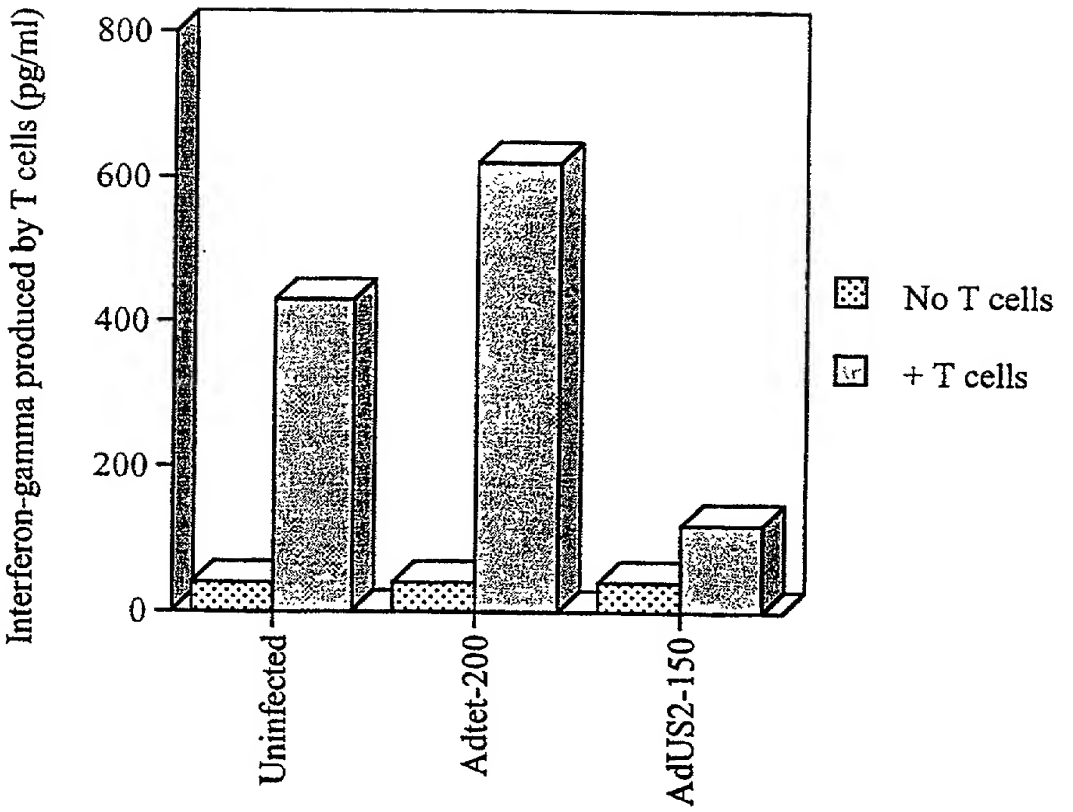

Interferon-gamma produced by T cells (pg/ml)
800
600
400
200
0
Uninfected
Adtet-200
AdUS2-150
No T cells
+ T cells

Fig. 6A

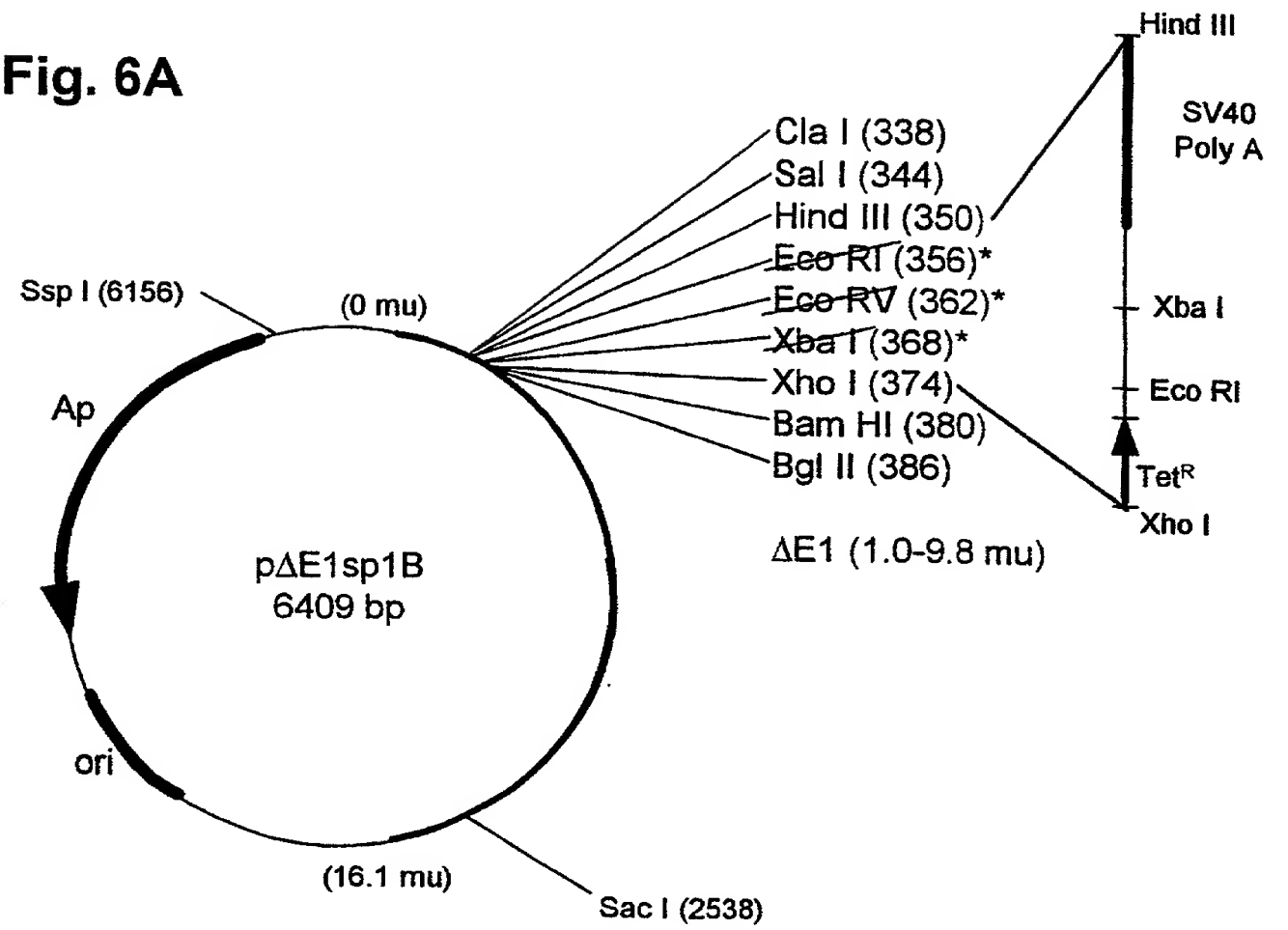

Hind III
SV40
Poly A
Cla I (338)
Sal I (344)
Hind III (350)
Eco RI (356)*
Eco RV (362)*
Xba I (368)*
Xho I (374)
Bam HI (380)
Bgl II (386)
Xba I
Eco RI
Tet^R
Xho I
ΔE1 (1.0-9.8 mu)
Ssp I (6156)
(0 mu)
Ap
pΔE1sp1B
6409 bp
ori
(16.1 mu)
Sac I (2538)

Fig. 6B

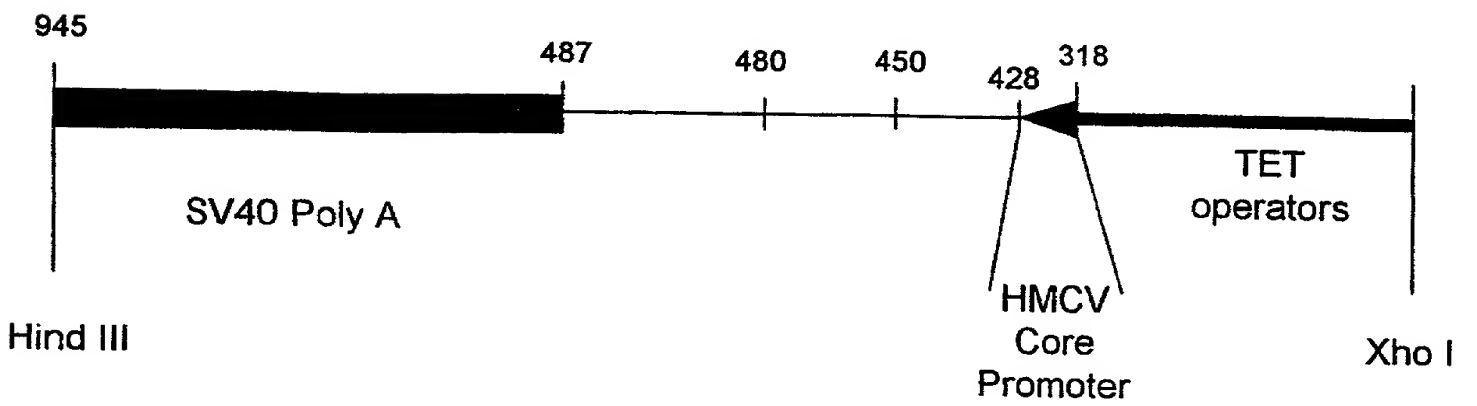

945
487
480
450
428
318
SV40 Poly A
TET
operators
HMCV
Core
Promoter
Hind III
Xho I

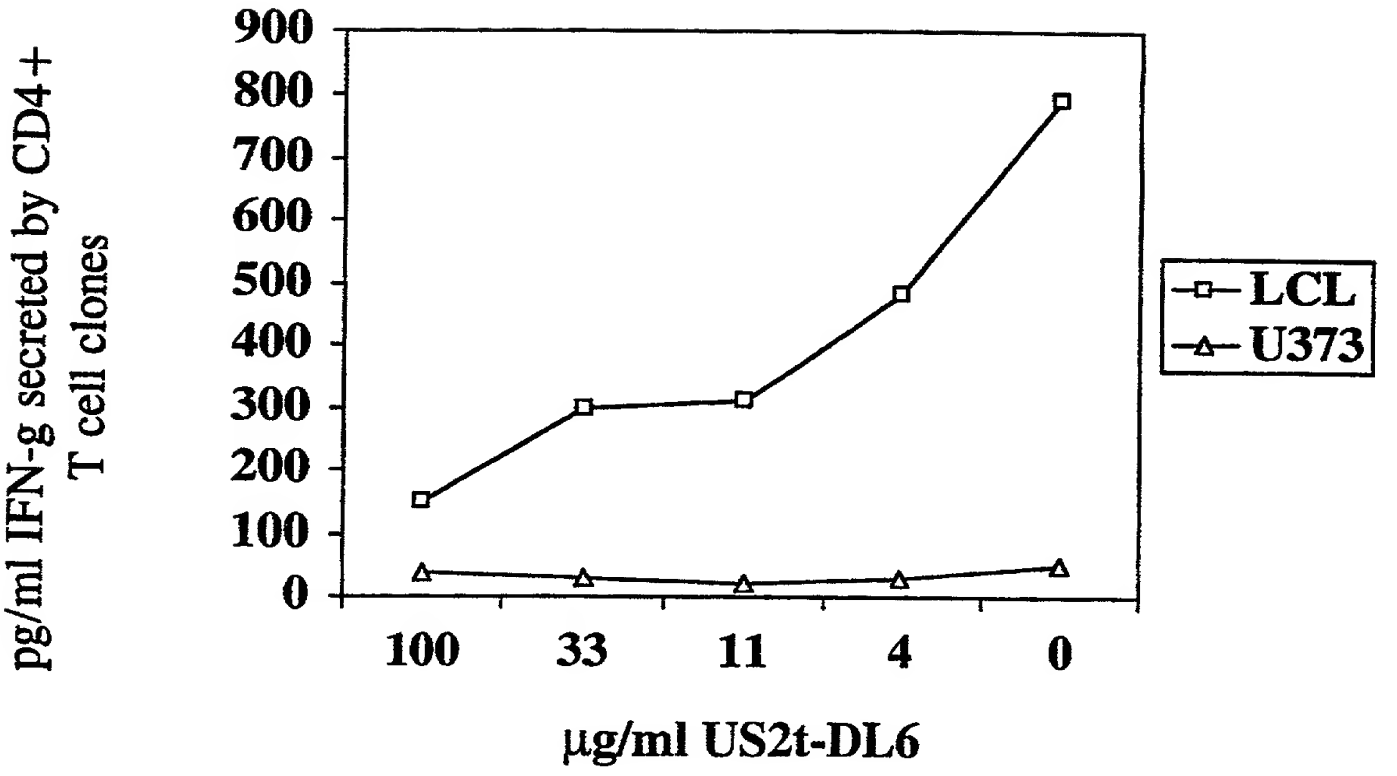
FIG. 7

```
      1                        25                                       50                                                                   100
US3   ~~~~MKPVLV LAILAVLF.. ..LRLADSVP RPLDVVV... .SEIRSAHFR VEENQCWFH. MGKLYFKGRM SGNFTEKHF. ..VNVGIVSQ SYMDRLQVSG
US2   MNNLWKAVVG LWTSMGPL.. ..IRLPDGIT KAGEDALRPW KSTAKHPWFQ IEDNRCYID. NGKLFARGSI VGNMSRFVFD PKADYGGVGE NLY..VHADD
US11  MNLVMLILAL WAPVAGSMPE LSLTLFDEPP PLVETEPLPP LSDVSEYRVE YSEARCVLRS GGRLEALWTL RGNLSVPTPT PRVYYQTL.E GYADRVPTP.
                                           *

                                 125                                                         175                   200
US3   EQYHHDERGA YFEWNIGGHP VTHTVDMVDI TLSTRWGDPK KYAACVPQVR MDYSS.QTIN WYLQRSMRDD NWGLLFRTLL VYLFSLVVLV LLTVGVS...
US2   VEFYPGESLK WNVRNLDVMP IFETLALRLV .....LQGDV IWLRCVPELR VDYTS.SAYM WNMQYGMVRK SYTHVAWTIV FYSINITLLV LFIVYVT...
US11  VEDV.SESLV AKRYWLRDYR VPQRTKLVLF YFSPCHQCQT YYVECEPRCL VPWVPLWSSL EDIERLLFED RRLMAYYALT IKSAQYTLMN VAVIQVFWGL
                                         *

      201                    218
US3   .A.......R LRFI~~~~
                                   } 58.6% SIMILAR, 22.9% IDENTICAL
US2   .VDCNLSMMW MRFFVC~~
                                   } 50.5% SIMILAR, 18.8% IDENTICAL
US11  YVKGWLHRHF PWMFSDQW
```

Fig. 8


pg/ml IFN-g secreted by CD4+ T cell clones
900
800
700
600
500
400
300
200
100
0
100
33
11
4
0
µg/ml US2t-DL6
LCL
U373

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS, the specification of which

☐ is attached hereto.

☒ was filed on August 1, 2001 as United States Application No. 09/890,806 .

☐ was filed on _____ as International Application No. _____.

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PCT/US00/02740	WIPO	2 February 2000	☐	☒
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

60/118,287	February 2, 1999
Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT

International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US00/02740	2 February 2000	Pending on August 1, 2001
(Application No.)	(Filing Date)	(Status: patented, Pending, abandoned)

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint the practitioners associated with the customer number provided below to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

Customer Number 24197

Name	Reg. No.	Name	Reg. No.
BUNKER, Gillian	47,461	ORR, David E.	44,988
BURG, Daniel B.	41,649	PETERSEN, David P.	28,106
CALDWELL, Lisa M.	41,653	POLLEY, Richard J.	28,107
CARLSON, Anne	47,472	RINEHART, Kyle B.	47,027
GIRARD, Michael P.	38,467	RUPERT, Wayne W.	34,420
HAENDLER, Jeffrey B.	43,652	RYBAK, Sheree L.	47,913
HARDING, Tanya M.	42,630	SCOTTI, Robert F.	39,830
JAKUBEK, Joseph T.	34,190	SIEGEL, Susan Alpert	43,121
JONCUS, Stephen J.	44,809	SLATER, Stacey C.	36,011
JONES, Michael D.	41,879	STEPHENS Jr., Donald L.	34,022
KLARQUIST, Kenneth S.	16,445	STUART, John W.	24,540
KLITZKE II, Ramon A.	30,188	VANDENBERG, John D.	31,312
LEIGH, James S.	20,434	WHINSTON, Arthur L.	19,155
MAURER, Gregory L.	43,781	WIGHT, Stephen A.	37,759
MIRHO, Charles A.	41,199	WINN, Garth A.	33,220
NOONAN, William D.	30,878		

Address all telephone calls to Tanya M. Harding, Ph.D. at telephone number (503) 226-7391.

Address all correspondence to:

Customer Number 24197

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the

application or any parent issued thereon.

Full Name of Sole or First Inventor: David C. Johnson

Inventor's Signature _____ 11-16-2001 Date

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Citizenship: Canada

Post Office Address: 7510 SW Kelly, Portland, Oregon 97219

Full Name of Second Inventor: Roman Tomazin

Inventor's Signature _____ Date

Residence: Burnaby, British Columbia, Canada

Citizenship: Canada

Post Office Address: 3953 Godwin Avenue, Apt. 305, Burnaby, BC, Canada V5G 4A1

Full Name of Third Inventor: Jessica Boname

Inventor's Signature _____ Date

Residence: Cambridge, United Kingdom

Citizenship: Canada

Post Office Address: 7 Ventress Cose, Cambridge, UK CB1 8QX

TMH:jlb 11/14/01 899-59399 64097

Full Name of Fourth Inventor: 4D Nagendra R. Hegde

Inventor's Signature _____ 11.15.2001 Date

Residence: Portland, Oregon OR

Citizenship: India

Post Office Address: 1011 SW Curry Street, Apt. 10, Portland, Oregon 97201

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS, the specification of which

☐ is attached hereto.

☒ was filed on August 1, 2001 as United States Application No. 09/890,806 .

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I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PCT/US00/02740	WIPO	2 February 2000	☐	☒
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

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60/118,287	February 2, 1999
Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT

International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US00/02740	2 February 2000	Pending on August 1, 2001
(Application No.)	(Filing Date)	(Status: patented, Pending, abandoned)

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

I hereby appoint the practitioners associated with the customer number provided below to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

Customer Number 24197 KSCLW

Name	Reg. No.	Name	Reg. No.
BUNKER, Gillian	47,461	ORR, David E.	44,988
BURG, Daniel B.	41,649	PETERSEN, David P.	28,106
CALDWELL, Lisa M.	41,653	POLLEY, Richard J.	28,107
CARLSON, Anne	47,472	RINEHART, Kyle B.	47,027
GIRARD, Michael P.	38,467	RUPERT, Wayne W.	34,420
HAENDLER, Jeffrey B.	43,652	RYBAK, Sheree L.	47,913
HARDING, Tanya M.	42,630	SCOTTI, Robert F.	39,830
JAKUBEK, Joseph T.	34,190	SIEGEL, Susan Alpert	43,121
JONCUS, Stephen J.	44,809	SLATER, Stacey C.	36,011
JONES, Michael D.	41,879	STEPHENS Jr., Donald L.	34,022
KLARQUIST, Kenneth S.	16,445	STUART, John W.	24,540
KLITZKE II, Ramon A.	30,188	VANDENBERG, John D.	31,312
LEIGH, James S.	20,434	WHINSTON, Arthur L.	19,155
MAURER, Gregory L.	43,781	WIGHT, Stephen A.	37,759
MIRHO, Charles A.	41,199	WINN, Garth A.	33,220
NOONAN, William D.	30,878		

Address all telephone calls to Tanya M. Harding, Ph.D. at telephone number (503) 226-7391.

Address all correspondence to:

Customer Number 24197 KSCLW

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the

application or any patent issued thereon.

Full Name of Sole or First Inventor: David C. Johnson

Inventor's Signature _____ Date _____

Residence: Portland, Oregon

Citizenship: Canada

Post Office Address: 7510 SW Kelly, Portland, Oregon 97219

Full Name of Second Inventor: Roman Tomazin

Inventor's Signature _____ Date Oct 14/01

Residence: Burnaby, British Columbia, Canada

Citizenship: Canada

Post Office Address: ~~3953 Godwin Avenue, Apt. 305, Burnaby, BC, Canada V5G 4A1~~
7050 Ramsay Ave, #A
Burnaby, B.C., Canada
V5E 3L2

Oct 14/01

Full Name of Third Inventor: Jessica Boname

Inventor's Signature _____ Date _____

Residence: Cambridge, United Kingdom

Citizenship: Canada

Post Office Address: 7 Ventress Cose, Cambridge, UK CB1 8QX

Full Name of Fourth Inventor: Nagendra R. Hegde

Inventor's Signature ______________________________ ______________

Date

Residence: Portland, Oregon

Citizenship: India

Post Office Address: 1011 SW Curry Street, Apt. 10, Portland, Oregon 97201

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS, the specification of which

☐ is attached hereto.

☒ was filed on August 1, 2001 as United States Application No. 09/890,806 .

☐ was filed on _____ as International Application No. _____.

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PCT/US00/02740	WIPO	2 February 2000	☐	☒
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code,§ 119(e) of any United States provisional application(s) listed below:

60/118,287	February 2, 1999
Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT

International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US00/02740	2 February 2000	Pending on August 1, 2001
(Application No.)	(Filing Date)	(Status: patented, Pending, abandoned)

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CARLSON, Anne	47,472	RINEHART, Kyle B.	47,027
GIRARD, Michael P.	38,467	RUPERT, Wayne W.	34,420
HAENDLER, Jeffrey B.	43,652	RYBAK, Sheree L.	47,913
HARDING, Tanya M.	42,630	SCOTTI, Robert F.	39,830
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JONCUS, Stephen J.	44,809	SLATER, Stacey C.	36,011
JONES, Michael D.	41,879	STEPHENS Jr., Donald L.	34,022
KLARQUIST, Kenneth S.	16,445	STUART, John W.	24,540
KLITZKE II, Ramon A.	30,188	VANDENBERG, John D.	31,312
LEIGH, James S.	20,434	WHINSTON, Arthur L.	19,155
MAURER, Gregory L.	43,781	WIGHT, Stephen A.	37,759
MIRHO, Charles A.	41,199	WINN, Garth A.	33,220
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application or any patent issued thereon.

Full Name of Sole or First Inventor: David C. Johnson

Inventor's Signature _____ _____
Date

Residence: Portland, Oregon

Citizenship: Canada

Post Office Address: 7510 SW Kelly, Portland, Oregon 97219

Full Name of Second Inventor: Roman Tomazin

Inventor's Signature _____ _____
Date

Residence: Burnaby, British Columbia, Canada

Citizenship: Canada

Post Office Address: 3953 Godwin Avenue, Apt. 305, Burnaby, BC, Canada V5G 4A1

Full Name of Third Inventor: Jessica Boname

Inventor's Signature Jessica Boname 19th Oct. 2001
Date

Residence: MELBOURN ~~Cambridge,~~ United Kingdom JB. 19.10.01

Citizenship: Canada

Post Office Address: ~~7 Ventress Cose, Cambridge, UK CB1 8QX~~ JB. 19.10.01
36 MEDCALFE WAY, MELBOURN, CAMBRIDGESHIRE, UK SG8 6HU

Full Name of Fourth Inventor: Nagendra R. Hegde

Inventor's Signature _____ _____
Date

Residence: Portland, Oregon

Citizenship: India

Post Office Address: 1011 SW Curry Street, Apt. 10, Portland, Oregon 97201

SEQUENCE LISTING

<110> Johnson *et al.*

<120> INHIBITION OF THE MHC CLASS II ANTIGEN PRESENTATION PATHWAY AND PRESENTATION TO CD4+ CELLS

<130> 0899-54203 Johnson

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<151> 1999-02-02

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ccc ttg atc cgc ctg ccc gat gga atc act aaa gcc ggg gaa gac gcg 96
Pro Leu Ile Arg Leu Pro Asp Gly Ile Thr Lys Ala Gly Glu Asp Ala
20 25 30

ctc cgg ccc tgg aag tcc acg gcc aag cat ccc tgg ttt caa atc gag 144
Leu Arg Pro Trp Lys Ser Thr Ala Lys His Pro Trp Phe Gln Ile Glu
35 40 45

gac aac cgg tgc tac att gac aac ggc aag ttg ttt gct cgg ggg agc 192

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Ile Val Gly Asn Met Ser Arg Phe Val Phe Asp Pro Lys Ala Asp Tyr
 65                  70                  75                  80

ggc ggc gtg gga gag aac ctg tac gta cac gcc gac gac gtg gag ttc   288
Gly Gly Val Gly Glu Asn Leu Tyr Val His Ala Asp Asp Val Glu Phe
                 85                  90                  95

gtt ccc ggg gag tcg tta aag tgg aac gtg cgg aac tta gat gtg atg   336
Val Pro Gly Glu Ser Leu Lys Trp Asn Val Arg Asn Leu Asp Val Met
            100                 105                 110

ccg atc ttc gag acg cta gcc ctg cgt ctg gta ctg caa ggg gat gtg   384
Pro Ile Phe Glu Thr Leu Ala Leu Arg Leu Val Leu Gln Gly Asp Val
        115                 120                 125

atc tgg ctg cgt tgc gtc ccc gaa ctg cga gtg gat tac acg tct agc   432
Ile Trp Leu Arg Cys Val Pro Glu Leu Arg Val Asp Tyr Thr Ser Ser
    130                 135                 140

gcg tac atg tgg aac atg cag tac ggg atg gtg cgg aag tca tac acg   480
Ala Tyr Met Trp Asn Met Gln Tyr Gly Met Val Arg Lys Ser Tyr Thr
145                 150                 155                 160

cat gtg gcc tgg aca ata gtg ttt tac tcc ata aac att acc ctg ttg   528
His Val Ala Trp Thr Ile Val Phe Tyr Ser Ile Asn Ile Thr Leu Leu
                165                 170                 175

gta ttg ttt atc gtg tat gtg act gtg gac tgt aac ttg tct atg atg   576
Val Leu Phe Ile Val Tyr Val Thr Val Asp Cys Asn Leu Ser Met Met
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Leu Arg Pro Trp Lys Ser Thr Ala Lys His Pro Trp Phe Gln Ile Glu
        35                  40                  45
Asp Asn Arg Cys Tyr Ile Asp Asn Gly Lys Leu Phe Ala Arg Gly Ser
    50                  55                  60
Ile Val Gly Asn Met Ser Arg Phe Val Phe Asp Pro Lys Ala Asp Tyr
65                  70                  75                  80
Gly Gly Val Gly Glu Asn Leu Tyr Val His Ala Asp Asp Val Glu Phe
                85                  90                  95
Val Pro Gly Glu Ser Leu Lys Trp Asn Val Arg Asn Leu Asp Val Met
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Pro Ile Phe Glu Thr Leu Ala Leu Arg Leu Val Leu Gln Gly Asp Val
        115                 120                 125
Ile Trp Leu Arg Cys Val Pro Glu Leu Arg Val Asp Tyr Thr Ser Ser
    130                 135                 140
Ala Tyr Met Trp Asn Met Gln Tyr Gly Met Val Arg Lys Ser Tyr Thr
145                 150                 155                 160
His Val Ala Trp Thr Ile Val Phe Tyr Ser Ile Asn Ile Thr Leu Leu
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Val Leu Phe Ile Val Tyr Val Thr Val Asp Cys Asn Leu Ser Met Met
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Trp Met Arg Phe Phe Val Cys
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        35                  40                  45

atc gag gac aac cgg tgc tac att gac aac ggc aag ttg ttt gct cgg  192
Ile Glu Asp Asn Arg Cys Tyr Ile Asp Asn Gly Lys Leu Phe Ala Arg
    50                  55                  60

ggg agc atc gtg ggc aac atg agt cgg ttc gtc ttc gat ccg aag gcc  240
Gly Ser Ile Val Gly Asn Met Ser Arg Phe Val Phe Asp Pro Lys Ala
65                  70                  75                  80

gat tat ggc ggc gtg gga gag aac ctg tac gta cac gcc gac gac gtg  288
Asp Tyr Gly Gly Val Gly Glu Asn Leu Tyr Val His Ala Asp Asp Val
                85                  90                  95

gag ttc gtt ccc ggg gag tcg tta aag tgg aac gtg cgg aac tta gat  336
Glu Phe Val Pro Gly Glu Ser Leu Lys Trp Asn Val Arg Asn Leu Asp
            100                 105                 110

gtg atg ccg atc ttc gag acg cta gcc ctg cgt ctg gta ctg caa ggg  384
Val Met Pro Ile Phe Glu Thr Leu Ala Leu Arg Leu Val Leu Gln Gly
        115                 120                 125
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gat gtg atc tgg ctg cgt tgc gtc ccc gaa ctg cga gtg gat tac acg   432
Asp Val Ile Trp Leu Arg Cys Val Pro Glu Leu Arg Val Asp Tyr Thr
    130                 135                 140

tct agc gcg tac atg tgg aac atg cag tac ggg atg gtg ggg cag cca   480
Ser Ser Ala Tyr Met Trp Asn Met Gln Tyr Gly Met Val Gly Gln Pro
145                 150                 155                 160

gaa ctc gcc ccg gaa gac ccc gag gat tcg gcc ctc ttg gag gac ccc   528
Glu Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro
                165                 170                 175

gtg tga                                                           534
Val
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Asp Ala Leu Arg Pro Trp Lys Ser Thr Ala Lys His Pro Trp Phe Gln
        35                  40                  45
Ile Glu Asp Asn Arg Cys Tyr Ile Asp Asn Gly Lys Leu Phe Ala Arg
    50                  55                  60
Gly Ser Ile Val Gly Asn Met Ser Arg Phe Val Phe Asp Pro Lys Ala
65                  70                  75                  80
Asp Tyr Gly Gly Val Gly Glu Asn Leu Tyr Val His Ala Asp Asp Val
                85                  90                  95
Glu Phe Val Pro Gly Glu Ser Leu Lys Trp Asn Val Arg Asn Leu Asp
            100                 105                 110
Val Met Pro Ile Phe Glu Thr Leu Ala Leu Arg Leu Val Leu Gln Gly
        115                 120                 125
Asp Val Ile Trp Leu Arg Cys Val Pro Glu Leu Arg Val Asp Tyr Thr
    130                 135                 140
Ser Ser Ala Tyr Met Trp Asn Met Gln Tyr Gly Met Val Gly Gln Pro
145                 150                 155                 160
Glu Leu Ala Pro Glu Asp Pro Glu Asp Ser Ala Leu Leu Glu Asp Pro
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Val
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